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- 64 Cloning and expression of HTLV-III DNA.
- The determination of the nucleotide sequence of HTLV-III DNA; identification, isolation and expression of HTLV-III sequences which encode immunoreactive polypeptides by recombinant DNA methods and production of viral RNA are disclosed. Such polypeptides can be employed in immunoassays to detect HTLV-III.

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#### Technical Fields

This invention is in the fields of molecular biology and virology and in particular relates to human T cell leukemia virus - type III (HTLV-III).

#### Background

The term human T cell leukemia-lymphoma virus (HTLV) refers to a unique family of T cell tropic retroviruses. These viruses play an important role in the pathogenesis of certain T cell neoplasms. There are presently three known types of HTLVs. One subgroup of the family, HTLV-type I (HTLV-I), is linked to the cause of adult T-cell leukemia-lymphoma (ATLL) that occurs in certain regions of Japan, the Caribbean and Africa. HTLV-type II (HTLV-II) has been isolated from a patient with a T-cell variant of hairy cell leukemia. M.Popovic et al., Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS. Science, 224:497-500 (1984).

HTLV-type III (HTLV-III) has been isolated from may patients with acquired immunodeficiency syndrome (AIDS). HTLV-III refers to prototype virus isolated from AIDS patients. Groups reported to be at greatest risk for AIDS include homosexual or bisexual males; intravenous drug users and Haitian immigrants to the United States. Hemophiliacs who receive blood products pooled from donors and recipients of multiple blood transfusions are also at risk. Clinical manifestations of AIDS include severe, unexplained immune deficiency which generally involves a depletion of helper T lymphocytes. These may be accompanied by malignancies and infections. The mortality rate for patients with AIDS is high Aless severe form of AIDS also exists, in which there may be lymphadenopathy and depressed helper T cell counts; there is not, however, the devastating illness characteristic of full-blown AIDS. There are many individuals, who are classified as having early AIDS (pre-AIDS), who exhibit these signs. It is not now possible to predict who among them will develop the more serious symptoms.

Much of the evidence implicates HTLV-III as the etiological agent of the infectious AIDS. First, there is consistent epidemiology; greater than 95% of the patients with AIDS have antibodies specific for HTLV-III. Second, there has been reproducible identification and isolation of virus in this disease; more than 100 variants of HTLV-III have been isolated from AIDS patients. Third, there has been transmission of the disease to normal healthy individuals who received blood transfusions from infected blood donors.

HTLV-III has been shown to share several properties with HTLV-I and HTLV-II but also to be morphologically, biologically and antigenically distinguishable. R.C. Gallo et al., Frequent Detection and Isola-

tion of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. Science, 224:500-501. (1984). For example, HTLV-III has been shown to be antigenically related to HTLV-I and HTLV-II by demonstrating cross-reactivity with antibodies to HTLV-I and HTLV-II core proteins, p24 and p19, and envelope antigens and by nucleic acid cross-hybridization studies with cloned HTLV-I and HTLV-II DNAs. However, unlike HTLV-I and HTLV-II, it lacked the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro, and has the cytopathic effect on infected cells only.

Like the RNA genome of other retroviruses, the RNA genome of HTLV-III contains three genes which encode viral proteins: 1) the gag gene, which encodes the internal structural (nucleocapsid or core) proteins; 2) the pol gene, which encodes the RNA-directed DNA polymerase (reverse transcriptase); and 3) the env gene, which encodes the envelope glycoproteins of the virion. In addition, the HTLV-III genome contains a region designated Px, located between the env gene and the 3' LTR, which appears to be involved in functional killing of the virus.

At this time, AIDS is still difficult to diagnose before the onset of clinical manifestations. There is no method presently available for the prevention of the disease. Treatment of those with AIDS is generally not successful and victims succumb to the devastating effects HTLV-III has on the body.

#### Summary of the Invention

This invention is based upon applicant's cloning of HTLV-III DNA in recombinant/vector host systems capable of expressing immunoreactive HTLV-III polypeptides. Based on the cloning of HTLV-III DNA in systems which express immunoreactive-polypeptides, applicant has developed methods useful in the diagnosis, treatment and prevention of AIDS. Applicant has developed methods of detecting HTLV-III and antibodies against HTLV-III in body fluids (e.g. blood, saliva, semen), and methods useful in immunotherapy (e.g. vaccination and passive immunization against AIDS). In addition, applicant has developed methods of making HTLV-III DNA probes and RNA probes useful in detecting HTLV-III in body fluids.

Polypeptides encoded by segments of the HTLV-III genome have been produced by these recombinant DNA methods. For example, polypeptides encoded by three regions of the HTLV-III genome (an env gene sequence, an env-lor gene sequence and a 1.1Kb EcoRI restriction fragment from HTLV-III cDNA) have been produced. The polypeptides expressed have been isolated. These polypeptides are immunoreactive with sera of patients having AIDS and with antibodies to HTLV-III and thus are useful in screening blood and other body fluids for the presence of an-

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tibodies against HTLV-III. Applicant's invention therefore provides a method not only for diagnosis AIDS, but also for preventing the transmission of the disease to others through blood or blood components harboring HTLV-III. The latter is particularly valuable in screening donated blood before it is transfused or used to obtain blood components (e.g., Factor VIII for the treatment of hemophilia; Factor IX)

Polypeptides produced by the recombinant DNA methods are employed in the production of antibodies, including monoclonal antibodies, against the virus. Such antibodies form the basis for immunoassay and diagnostic techniques for directly detecting HTLV-III,in body fluids such as blood, saliva, semen, etc. Neutralizing antibodies against the virus may be used to passively immunize against the disease.

Applicant's cloning of HTLV-III DNA in such recombinant vector host systems also provides the basis for determination of the nucleotide sequence of HTLV-III DNA. The DNA probes are homologous to DNA regions which are unique to the HTLV-III genome. DNA probes provide another method of detecting HTLV-III in blood, saliva or other body fluids. RNA probes which contain regions unique to the HTLV-III genome can also be formed and used for the detection of HTLV-III in body fluids.

#### **Brief Description of the Figures**

Figure 1 is a representation of HTLV-III DNA. figure 1a shows sites at which the genome is cut by the restriction enzyme Sstl and Figure 1b shows the fragments of HTLV-III genome produced through the action of restriction enzymes Kpn, EcoRI and Hind III.

Figure 2 is a representation of HTLV-III DNA. Figure 2a shows the location of restriction enzyme sites in the genome and Figure 2b shows the location in the HTLV-III genome of DNA inserts in open rending frame clones. The (+) and (-) indicate reactivity and lack of reactivity, respectively, of the fusion protein expressed by cells transformed by the ORF vectors with sera of AIDS patients.

Figure 3 shows the nucleotide sequence for HTLV-III DNA and the predicted amino acid sequence of the four longest open reading frames. Restriction enzyme sites are indicated above the nucleotide sequence.

Figure 4 is an immunoblot showing the position on an SDS polyacrylamide gel of HTLV-III <u>env-Beta-galactosidase</u> fusion proteins.

Figure 5 shows sites at which the genome is cut by the restriction enzyme EcoRI and construction of recombinant plasmids carrying HTLV-III DNA.

Figure 6 is an immunoblot showing the positions on nitrocellulose blots of peptides produced by bacterial cells transformed by recombinant construct ompA1-R-6; ompA2-R-7 and ompA3-R-3, into which a 1.1Kb EcoRI HTLV-III cDNA restriction fragment had

been inserted. Figure 6a shows the nucleotide sequence of the ompA signal peptide and the pertinent region of recombinant plasmids ompA1-R-6; ompA2-R-7 and ompA3-R-3.

Figure 7 is an immmunoblot showing blocking of reaction between HTLV-III antigens and an AIDS serum by lysates of <u>E.coli</u> containing HTLV-III DNA recombinant plasmid ompA1-R-6 (lanes 1-5) and no blocking of the reaction by lysates of <u>E.coli</u> control cells (lanes 6-10).

Figure 8 is an immunoblot showing the presence or absence of antibodies against the peptide encoded by the 1.1Kb EcoRI HTLV-III restriction fragment of HTLV-III cDNA in sera from healthy individuals (lanes 1-3) and from AIDS patients (lanes 4-11). Purified HTLV-III virus (panel A) or total cell lysate of bacterial clone ompA1-R-6(O1R6) were reacted with sera samples.

Figure 9 represents the open reading frame expression vector pMRIOO having HTLV-III DNA.

Figure 10 represents lambdaCI-HTLV-III betagalactosidase fusion proteins. Figure 10a is an immunoblot showing the position on SDS polyacrylamide gel of lambdaCI-HTLV-III beta-galactosidase fusion proteins, and Figure 10b shows the immunoreactivity of such proteins with sera from AIDS patients.

#### Best Mode of Carrying Out the Invention

Despite the similarity between HTLV-III and the other members of the HTLV-bovine leukemia virus (BLV) family of viruses, the biology and pathology of HTLV-III differs substantially. For example, relatively little homology has been found in the HTLV-III genome when compared with that of the HTLV-I or -II genome. Infection with HTLV-III often results in profound immunosuppression (AIDS), consequent to the depletion of the OKT4(+) cell population. This effect is mirrored by a pronounced cytopathic, rather than transforming, effect of HTLV-III infection upon the OKT4(+) cells in lymphocyte cultures in vitro. In contrast, infection with HTLV-I results in a low incidence of T-cell leukemia lymphoma (an OKT4(+) cell malignancy). There is evidence for some degree of immunodeficiency in HTLV-I patients as well. Infection of primary lymphocytes in culture by HTLV-I and -II results in vitro transformation of predominantly OKT4(+) cells. A cytopathic effect of HTLV-I infection upon lymphocytes is apparent, but the effect is not as pronounced as that observed for HTLV-III.

HTLV-III also differs from HTLV-I and -II in the extent of infectious virion production in vivo and in vitro. High titers of cell free, infectious virions can be obtained from AIDS patient semen and saliva and from the supernatant of cultures infected with HTLV-III. Very few, if any, cell free infectious virions can be recovered from adult T-cell leukemia lymphoma (ATLL) patients or from cultures infected with HTLV-I or -II.

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Envelope glycoprotein is the major antigen recognized by the antiserum of AIDS patients. In this respect, HTLV resembles other retroviruses, for which the envelope glycoprotein is typically the most antigenic viral polypeptide. In addition, the neutralizing antibodies are generally directed toward the envelope glycoprotein of the retrovirus. Serum sample from 88 percent to 100 percent of those with AIDS have been shown to have antibodies reactive with antigens of HTLV-III; the major immune reactivity was directed against p41, the presumed envelope antigen of HTLV-III. Antibodies to core proteins have also been demonstrated in serum of AIDS patients, but do not appear to be as effective an indicator of infection as is the presence of antibodies to envelope antigen.

The p41 antigen of HTLV-III has been difficult to characterize because the viral envelope is partially destroyed during the process of virus inactivation and purification. This invention responds to the great need to characterise this antigenic component of the HTLV-III virus and to determine the existence and identity of other viral antigenic components in several ways. It provides products, such as HTLV-III polypeptides, antibodies to the polypeptides and RNA and DNA probes, as well as methods for their production. These serve as the basis for screening, diagnostic and therapeutic products and methods.

This invention relates to HTLV-III polypeptides which are produced by translation of recombinant DNA sequences encoding HTLV-III proteins. Polypeptides which are produced in this way and which are immunoreactive with serum from. AIDS patients or antibodies to HTLV-III are referred to as recombinant DNA-produced immunoreactive HTLV-III polypeptides. They include, but are not limited to, antigenic HTLV-III core and envelope polypeptides which are produced by translation of the recombinant DNA sequences specific to the gag and the env DNA sequences encoding HTLV-III core proteins and envelope glycoproteins, respectively. They also include the polypeptides which are produced by translation of the recombinant DNA sequences included in a 1.1Kb EcoRI restriction fragment of HTLV-III cDNA and recombinant DNA sequences specific to the sor gene and the Px genes of HTLV-III. The sor DNA sequence is common to replication competent HTLV-III viruses. The Px genes contain a coding sequence with one large open reading frame (lor), located between the env gene and the 1' end of the HTLV-III genome. Both the env DNA sequences and the lor DNA sequences are located within the same open reading frame of the HTLV-III genome and this gene region is accordingly designated env-lor.

The polypeptides encoded by these regions of the HTLV III can be used in immunochemical assays for detecting antibodies against HTLV-III and HTLV-VIII infection. These methods can assist in diagnosing AIDS. In addition, they can also be employed to

screen blood before it is used for transfusions or for the production of blood components (e.g., Factor VIII for the treatment of hemophilia). Availability of screening technics will reduce the risk of AIDS transmission

Detection of antibodies reactive with the polypeptides can be carried out by a number of established methods. For example, an immunoreactive HTLV III polypeptide can be affixed to a solid phase (such as polystyrene bead or other solid support). The solid phase is then incubated with blood sample to be tested for antibody against HTLV-III. After an appropriate incubation period the solid phase and blood sample are separated. Antibody bound to the solid phase can be detected with labeled polypeptide or with a labeled antibody against human immunoglobulin.

HTLV-III polypeptides can be used in a vaccine prevention of AIDS. For vaccination against the virus, immunogenic polypeptides which elicit neutralizing antibody would be employed. The leading candidates for use in vaccines are the viral envelop polypeptides.

The polypeptides can also be used to produce antibodies, including monoclonal antibodies, against the HTLV-III polypeptides. These antibodies can be used in immunochemical assays for direct detection of the virus in body fluids (such as blood, saliva and semen). Assays employing monoclonal antibody against specific HTLV III antigenic determinants will reduce false-positive results thereby improving accuracy of assays for the virus Antibodies against the virus may also be useful in immunotherapy. For example, antibodies may be used to passively immunize against the virus.

The methods of producing the polypeptides are also a subject of this invention, as are diagnostic methods based on these polypeptides.

This invention also provides methods for the isolation of genes of HTLV-III which encode immuno-reactive polypeptides; identification of the nucleotide sequence of these genes; introduction of DNA sequences specific to these viral DNA sequences into appropriate vectors to produce viral RNA and the formation of DNA probes. These probes are comprised of sequences specific to HTLV-III DNA and are useful, for example, for detecting complementary HTLV-III DNA sequences in body fluids (e.g., blood).

#### HTLV-III POLYPEPTIDES

Genetic engineering methods are used to isolate segments of HTLV-III DNA which encode immunor-eactive HTLV-III polypeptides. Among these are polypeptides which are immunoreactive with serum from AIDS patients or antibodies to HTLV-III. These polypeptides include the core protein, a 15Kd peptide encoded by a 1.1K.b EcoRI HTLV-III restriction fragment of HTLV-III DNA and the envelope glycoprotein. These methods are also used to sequence the frag-

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ments which encode the polypeptides. The proviral genes integrated into host cell DNA are molecularly cloned and the nucleotide sequences of the cloned provirus is determined.

An <u>E. coli</u> expression library of HTLV-III DNA is constructed. The HTLV-III genome is cloned and cuts are then made in the cloned HTLV-III genome with restriction enzymes to produce DNA fragments. (Figures 1 and 2) HTLV-III DNA fragments of approximately 200-500bp are isolated from agarose gel, end repaired with T<sub>4</sub> polymerase and ligated to linker DNA. The linker ligated DNA is then treated with a restriction enzyme, purified from agarose gel and cloned in an expression vector. Examples of the expression vectors used are: OmpA, pIN (A,B and C), lambda pL, T7, lac, Trp, ORF and lambda gt11. In addition, mammalian cell vectors such as pSV28pt, pSV2neo, pSVdhfr and VPV vectors, and yeast vectors, such as GALI and GAL10, may be used.

The bacterial vectors contain the lac coding sequences, into which HTLV-III DNA can be inserted for the generation of B-galactosidase fusion protein. The recombinant vectors are then introduced into bacteria (e.g., E.coli); those cells which take up a vector containing HTLV-III DNA are said to be transformed. The cells are then screened to identify cells which have been transformed and are expressing the fusion protein. For example, the bacteria are plated on MacConkey agar plates in order to verify the phenotype of clone. If functional B-galactosidase is being produced, the colony will appear red.

Bacterial colonies are also screened with HTLV-III DNA probes to identify clone: containing the DNA regions of interest (e.g., HTLV-III gag pol and env DNA sequences). Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

This identification of cells harboring the HTLV-III DNA sequences makes it possible to produce HTLV-III polypeptides which are immunoreactive with HTLV-III specific antibody. The cells from the selected colonies are grown in culture under conditions allowing the expression of the hybrid protein. Cell protein is then obtained by means known in the art. For example, the culture can be centrifuged and the resulting cell pellet broken. Polypeptides secreted by the host cell can be obtained (without disruption of the cells) from the cell culture supernatant.

The total cellular protein is analyses by being run on an SDS polyacrylamide gel electrophoresis. The fusion proteins are identified at a position on the gel which contains no other protein. Western blot analyses are also carried out on the clones which screened positive. Such analyses are performed with serum from AIDS patients, with the result that it is possible to identify those clones expressing HTLV-III B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody. Lambda 10 clones

harboring HTLV-III DNA are cloned from the replicated form of the virus. As the retrovirus is replicating, double stranded DNA is being produced. The cloned HTLV-III DNA is digested with the restriction enzyme Sstl. (Figure 1a) Because there are two Sstl recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA. sequence removed from the lambda<sub>10</sub> vector. As a result, a small (approximately 200 bp) fragment of the HTLV-III DNA is missing.

The resulting DNA is linearized and fragments are produced by digesting the linearized genomic DNA spanning the <a href="mailto:env">env</a> gene region with restriction enzymes. For example, fragments are produced using Kpn or EcoRl plus HindIII, as shown in Figure 1b. The resulting 2.3kb KpnI-KpnI fragments; 1.0kbEcoRl-EcoRl fragments and 2.4 EcoRl-HindIII fragments are solated by gel electrophoresis and electro-elution. These fragments are randomly sheared to produce smaller fragments. The fragments thus produced are separated from agarose gel and DNA fragments between about 200-500 are eluted.

The eluted 200-500bp DNA fragments are end filled through the use of E. coli T4 polymerase and blunt end ligated into an open reading frame expression (ORF) vector, such as pMRIOO. This ligation may occur at the Smal site of the pMRIOO vector, which contains two promoter regions, hybrid coding sequences of lambdaCl gene and lacl-LacZ gene fusion sequence. In the vector, these are out of frame sequences; as a result, the vector is nonproductive. The HTLV-III DNA is inserted into the vector, the correct DNA fragments will correct the reading frame, with the result that CI-HTLV-III-B-galactosidase fusion proteins are produced. The expression of the hybrid is under the control of the lac promoter. Based on the sequence of pMR100, it appears that if a DNA fragment insert cloned into the Smal site is to generate a proper open reading frame between the lambdaCI gene fragment and the lac-Z fragment, the inserted DNA must not contain any stop codons in the reading frame set by the frame of the lambdaCl gene.

The recombinant pMR100 vectors are than introduced into E. coli. The bacteria are plated on MacConkey agar plates to verify the phenotype of the clone. If functional B-galactosidase is being produced, the colomly will appear red. The colonies are also screened with HTLV-III DNA probes, for the purpose of identifying those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

The cells from the selected colonies are grown in culture. The culture is spun down and the cell pellet broken. Total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 4)

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Western blot analyses are also carried out on the clones which screened positive. Sera from AIDS patients are used, thus making it possible to identify those clones which express the HTLV-III-D D-galactosidase fusion proteins that cross-react with the HTLV-III specific antibody.

1000 clones were screened by this method; 6 were positive.

Because of the nature of the pMR100 cloning vehicle, a productive DNA insert should also be expressed as a part of a larger fusion polypeptide. HTLV-III env gene containing recombinant clones was identified by colony hybridization. The production of larger fusion polypeptides bearing functional B-galactosidase activity was verified by phenotype identification on MacConkey agar plates; by B-galactosidase enzymatic assays and by analysis on 75% SDS-polyacrylamide gels. Immunoreactivity of the larger protein with antibody to HTLV-III was assessed by western blot analysis using serum from AIDS patients. These large fusion proteins also reacted with anti-Bgalactosidase and anti-CI antiserum. This finding is consistent with the hypothesis that they are proteins of CI-HTLV-III-laciZ.

The open reading frame insert fragment of HTLV-III is further analyzed by DNA sequencing analysis. Because one of the two BamHI sites flanking the Smal cloning site in pMR100 is destroyed in the cloning step, positive clones are digested with restriction enzymes HindIII and clal to liberate the inserted HTLV-III DNA fragment. The HTLV-III ORF inserts are isolated from the fusion recombinant and cloned into M13 sequencing cloning vector mp18 and mp19 digested with HindIII and Accl. DNA sequences of the positive ORF clones are then determined.

Fragments of HTLV-III DNA of approximately 200-500 bps are isolated from agarose gel, end repaired with T<sub>4</sub> polymerase and ligated to EcoRI linker. The EcoRI linker ligated DNA is then treated with EcoRI purified from 1% agarose gel and cloned in an expression vector, lambda gt11. This vector contains lac Z gene coding sequences into which the foreign DNA can be inserted for the generation of B-galactosidase fusion protein. The expression of the hybrid gene is under the control of lac repressor. The lac repressor gene, lac I, is carried on a separate plasmid pMC9 in the host cell, E. coli Y1090. AIDS patient serum was used to probe the lambdagt11 library of HTLV-III genome DNA containing 1.5x104 recombinant phage. In a screen of 5000 recombinants, 100 independent clones that produced strong signals were isolated. The positive recombinant DNA dones were further characterized for their specific gene expression. Rabbit hyperimmune serum against P24 was also used to identify the gag gene specific clones. Nick-translated DNA probes of specific HTLV-III gene, specifically the gag gene, env gene and Px gene were used to group the positive immunoreactive clones into specific gene

region.

Recombinant clones that produced strong signals with AIDS serum and contain insert DNA spanning the HTLV-III gag, pol, sor and env-lor gene regions were examined in detail by mapping their insert with restriction enzymes and DNA sequencing analysis

# Determination of the Nucleotide Sequence of HTLV-III DNA

Genetic engineering methods are used to determine the nucleotide sequence of HTLV-III DNA. One technique that can be used to determine the sequence is a shotgun/random sequencing methods. HTLV-III DNA is sheared randomly into fragments of about 300-500 bp in size. The fragments are cloned, for example, using ml3, and the colonies screened to identify those having an HTLV-III DNA fragment insert. The nucleotide sequence is then generated, with multiple analysis producing overlaps in the sequence. Both strands of the HTLV-III DNA are sequenced to determine orientation. Restriction mapping is used to check the sequencing data generated.

The nucleotide sequence of one cloned HTLV-III genome (BH10) is shown in Figure 3, in which the position of sequences encoding gag protein p17 and the N-terminus of gag p24 and the C-terminus of gag p15 (which overlaps with the N-terminus of the pol protein) are indicated. The open reading frames (ORF) for pol, sor and env-lor are also indicated. The sequence of the remaining 182 base pairs of the HTLV-III DNA not present in clone BH10 (including a portion of R, U5, the tRNA primer binding site and a portion of the leader sequence) was derived from clone HXB2. The sequences of two additional clones (BH8 and BH5) are also shown. Restriction enzyme sites arc listed above the nucleotide sequence; sites present in clone BH8 but not in clone BH10 are in parentheses. Deletions are noted ([]) at nucleotides 251, 254, 5671 and 6987-7001. The nucleotide positions (to the right of each line) start with the transcriptional initiation site. The amino acid residues are numbered (to the right of each line) for the four largest open rending frames starting after the preceding termination codon in each case except gag which is enumerated from the first methionine codon. A proposed peptide cleavage site (V) and possible asparagine-linked glycosylation sites are shown (\*) for the env-lor open rending frame. The sequences in the LTR derived from clones BH8 and bH10 listed in the beginning of the figure are derived from the 3'-portion of each clone and are assumed to be identical to those present in the 5'-LTR of the integrated copies of these viral genomes.

Clone HXB2 was derived from a recombinant phage library of Xbal digested DNA from HTLV-III infected H9 cells cloned in lambdaJ1. H9 cells are hu-

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man leukemic cells infected by a pool of HTLV-III from blood of AIDS patients, F. Wong-Staal, Nature, 312, November, 1984. Cloning vector clones BH10, BH8, and BH5 were derived from a library of Sstl digested DNA from the Hirt supernatant fraction of HTLV-III infected H9 cells cloned in lambdagtWes.lambdaB. Both libraries were screened with cDNA probe synthesized from virion DNA using oligo.dT as a primer. Clones BH8, BH5, and a portion of HXB2 were sequenced as described by Maxam and Gilbert. (1980) Maxam, A. M. and Gilbert, Co. Methods in Enzymology. 65: 499-560. Clone BH10 was sequenced by the method of Sanger modified by the use of oligonucleotides complementary to the M13 insert sequence as primers and using Klenow fragment of DNA polymerase I or reverse transcriptase as the polymerase.

## Formation of RNA, RNA Probes and DNA Probes Specific to HTLV-III

DNA sequences which are an entire gene or segment of a gene from HTLV-III are inserted into a vector, such as a T7 vector. In this embodiment, the vector has the Tceu promoter from the T cell gene 10 promoter and DNA sequences encoding eleven amino acids from the T cell gene 10 protein.

The vectors are then used to transform cells, such as <u>E. coli</u>. The T7 vector makes use of the T7 polymerase, which catalyzes RNa formation and recognizes only T7 promoter, which is the site where RNA polymerase binds for the initiation of transcription. The T7 polymerase does not recognize <u>E. coli</u> promoter. As a result, if HTLV-III DNA sequences are inserted after the promoter and polymerase genes of the T7 vector, which recognizes them to the exclusion of the signals, and a terminator is placed immediately after the HTLV-III DNA sequences, the T7 vector will direct manufacture RNA complementary to the HTLV-III insert.

Determination of the nucleotide sequence of HTLV-III DNA also provides the basis for the formation of DNA probes. Both RNA proves and DNA HTLV-III probes must have a distinctive region of the HTLV-III genome in order to be useful in detecting HTLV-III in body fluids. There is relatively little homology between the HTLV-III genome and the HTLV-I and -II genomes and probes contain regions which are unique to HTLV-III (i.e., not shared with HTLV-I or -II). For example, nucleotide sequences in the env gene region of HTLV-III can be used.

Either viral RNA or DNA can be used for detecting HTLV-III in, for example, saliva, which is known to have a very high concentration of the virus. This can be tone, for example, by means of a dot blot, in which the saliva sample is denatured, blotted onto paper and then screened using either type of probe. If saliva is used as the test fluid, detection of HTLV-III is considerable faster and easier than is the case if blood

is tested.

# Production of Monoclonal Antibodies Reactive with HTLV-III Polypeptides

Monoclonal antibodies reactive with HTLV-III polypeptides are produced by antibody-producing cell lines. The antibody-producing cell lines may be hybridoma cell lines commonly known as hybridomas. The hybrid cells are formed by fusion of cells which produce antibody to HTLV-III polypeptide and an immortalizing cell, that is, a cell which imparts long term tissue culture stability on the hybrid cell. In the formation of the hydrid cell lines, the first fusion partner the antibody-producing cell - can be a spleen cell of an animal immunized against HTLV-III polypeptide. Alternatively, the antibody-producing cell can be isolated B lymphocyte which produces antibody against an HTLV-III antigen. The lymphocyte can be obtained from the spleen, peripheral blood, lymph nodes or other tissue. The second fusion partner - the immortal cell - can be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibodyproducing cell but also malignant.

Murine hybridomas which produce monoclonal antibodies against HTLV-III polypeptide are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against the polypeptide. To immunize the mice, a variety of different immunization protocols may be followed. For instance mice may receive primary and boosting immunizations of the purified polypeptide. The fusions are accomplished by standard procedures. Kohler and Milstein, (1975) Nature (London) 256, 495-497; Kennet, R., (1980) in Monoclonal Antibodies (Kennet et al., Eds. pp. 365-367, Plenum Press, NY).

The hydridomas are then screened for production of antibody reactive with the polypeptide. This can be performed by screening procedures known in the art.

Another way of forming the antibody-producing cell line is by transformation of antibody-producing cells. For example, a B lymphocyte obtained from an animal immunized against HTLV-III polypeptide may be infected and transformeed with a virus such as the Epstein-Barr virus in the case of human B lymphocytes to give an immortal antibody-producing cell. See, e.g., Kozbor and Rodor (1983) Immunology Today 4(3), 72-79. Alternaltively, the B lymphocyte may be transformed by a transforming gene or transforming gene product.

The monoclonal antibodies against HTLV-III polypeptide can be produced in large quantities by injectin, antibody-producing hybridomas into the peritoneal cavity of mice and, after an appropriate time, harvesting the ascites fluid which contains very high titer of homogenous antibody and isolating the monoclonal antibodies therefrom. Xenogeneic hybridomas should be injected into irradiated or athymic nude

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mice. Alternatively, the antibodies may be produced by culturing cells which produce HTLV-III polypeptide in vitro and isolating secreted monoclonal antibodies from the cell culture medium. The antibodies produced according to these methods can be used in diagnostic assays (e.g., detecting HTLV-III in body fluids) and in passive immunotherapy. The antibodies reactive with HTLV-III polypeptides provide the basis for diagnostic tests for the detection of AIDS or the presence of HTLV-III in biological fluids (e.g., blood, semen, saliva) and for passive immunotherapy. For example, it is possible to produce anti p 41, to attach it to a solid phase using conventional techniques and to contact the body fluid to be tested with the immobilized antibody. In this way, HTLV-III (antigen) can be detected in the body fluid; this method results in far fewer false positive test results than do tests, in which antibody against HTLV-III is detected.

This invention will now be further illustrated by the following examples.

#### **EXAMPLE 1**

# PREPARATION OF SONICATED DNA FRAGMENTS

10 ug of gel purified HTLV-III restriction fragments were sonicated to fragment size on average of 500 bps. After sonication, the DNA was passed through a DEAE-cellulose column in 0.1XTBE in order to reduce the volume. The DEAE-bound DNA was washed with 5 ml of 0.2 M NaCI-TE (2 M NaCI, 10 mm Tris HCI pH 7.5, 1 mM EDTA) and then eluted with 1 M NaCl-TE, and ethanol precipitated. The size range of the sonicated DNA was then determined on 1.2% agarose gel. DNA fragments of desired length (200-500 bps) was eluted from the gel. T4 DNA polymerase was used to fill in and/or trim the single strand DNA termini generated by the sonication procedure. DNA fragments were incubated with T4 polymerase in the absence of added nucleotides for five minutes at 37°C to remove nucleotides from 3' end and then all 4 nucleotide precursors were added to a final concentration of 100 uM and the reaction mixture was incubated another 30 minutes to repair the 5'-end single stranded overhang. The reaction was stopped by heat inactivation of the enzyme at 68°C for 10 minutes. DNA was phenol extracted once, ethanol precipitated and resuspended in TE.

#### **EXAMPLE 2**

# CLONING OF RANDOM SHEARED DNA FRAGMENTS

The sonicated blunt end repaired HTLV-III DNA fragments were ligated into the Smal site of the ORF expression vector pMR100 and transformed into host

cell LG90 using standard transformation procedures. B-galactosidase positive phenotype of the transformant were identified by plating the transformed cell on ampicillin (25 ug/ml) containing McConkey agar plates and scoring the phenotype after 20 hours at 37°C.

#### **EXAMPLE 3**

#### HYBRID PROTEIN ANALYSIS

Ten milliliter samples of cells from an overnight saturated culture grown in L broth containing ampicillin (25 ug/ml) were centrifuged, the cell pellet was resuspended in 500 ul of 1.2 fold concentrated Laemmli sample buffer. The cells were resuspended by vortexing and boiling for 3 minutes at 100°C. The lysate was then repented by being forced through a 22 gunge needle to reduce the lysate viscosity. Approximately 10 ul of the protein samples were electrophoresed in 7.5% SDS-PAGE (SDS-polyacrylamide) gels.

Electrophoretic transfer of proteins from SDS-PA-GE gels to nitrocellulose paper was carried out according to Towbin et. al.. After the transfer, the filter was incubated at 37°C for two hours in a solution of 5% (w/v) nonfat milk in PBS containing 0.1% antifoam A and 0.0001% merthiolate to saturate all available protein binding sites. Reactions with AIDS antisera were carried out in the same milk buffer containing 1% AIDS patient antisera that had been preabsorbed with E. coli lysate. Reactions were performed in a sealed plastic bag at 4°C for 18-24 hours on a rotary shaker. Following this incubation, the filter was washed three times for 20 minutes each at room temperature in a solution containing 0.5% deoxycholic, 0.1 M NaCl, 0.5% triton X-100; 10 mm phosphate buffer pH 7.5 and 0.1 mM PMSF.

To visualize antigen-antibody interactions, the nitrocellulose was then incubated with the second goat antihuman antibody that had been iodinated with <sup>125</sup>I. The reaction with the iodinated antibody was carried out at room temperature for 10 minutes in the came milk buffer as was used for the first antibody. The nitrocellulose was then washed as previously described and exposed at -70°C using Kodak XAR5 film with an intensifying screen.

#### **EXAMPLE 4**

## SCREENING OF THE HTLV-III ORF LIBRARY BY COLONY HYBRIDIZATION

E. coli LG90 transformants were screened with HTLV-III DNA probes containing the DNA regions of interest (e.g. HTLV-III gag, env or Px gene specific sequences). Colonies were grown on nitrocellulose filter and screened according to the procedure of Grunsteir and Hogness by using a nick-translated HTLV-III

DNA as hybridization probe.

The DNA fragment was in general excised by restriction endonuclease digestion, gel purified, and <sup>32</sup>p-labeled to a specific activity of 0.5x10<sup>8</sup> cpm/ug by nick-translation (Rigby, P.W.J. et al., J. Mol. Biol. 113, 237 (1977). Duplicate nitrocellulose filters with DNA fixed to them were prehybridized with 6xSSC (0.9 M NaCl/0.09 M sodium citrate, pH 7.0), 5X Denhardt's solution (Denhardt's solution: 0.02% each of polyvinylpyrrolidone, Ficoll and bovine serum albumin) 10 ug of denatured sonicated E. coli DNA per ml at 55°C for 3-5 hours. The filters were then placed in a fresh sample of the same solution to which the denatured hydridization probe had been added. Hydridization was permitted to take place at 68°C for 16 hours. The filters were washed repeatedly in 0.3XSSC at 55°C. and then

#### **EXAMPLE 5**

# RECOMBINANT DNA PRODUCED PEPTIDE OF HTLV-III WHICH IS IMMUNOREACTIVE WITH SERA FROM PATIENTS WITH AIDS

An expression vector, pIN-III-ompA (ompa) was used. ompA has the lipoprotein (the most abundant protein in E.coli) gene promoter (Ipp) and the lacUV5 promoter-operator (Figure 1). ompA vectors also contain the DNA segment encoding the lac repressor, which allows the expression of the inserted DNA to be regulated by lac operon inducers such as IPTG. The ompA cloning vehicles contain three unique restriction enzyme sites Ecorl, HindIII, Bam HI in all three reading frames and permit the insertion of DNA into and of these restriction sites.

Various restriction fragments were excised from the recombinant done, lambdaBH10, which contains a 9 Kb long HTLV-III DNA insert in the Sstl site of the vector lambdagtWES lambdaB. These restrictions fragments were them inserted into the ompA vectors at all three reading frames and used to transform E. coli JA221 cells. Transformants were first screened for HTLV-III DNA by in situ colony hydridization using nick-translated HTLV-III DNA probes. The positive clones were then screened for expression of HTLV-III antigenic peptides using HTLV-III specific antibodies. For this, lysates of E. coli cell containing HTLV-III DNA recombinant plasmids were electrophoresis on 12.5% SDS-polyacrylamide gel and electroblotted onto nitrocellulose filters. The filters were then incubated first with well-characterized sera from AIDS patients and next with 1251-labelled goat anti-human IgG antibodies. The washed filters were autoradiographed to identify peptides reactive with anti-HTLV-III antibodies.

Several gene segments that encode-peptides showing immunoreactivity with anti-HTLV-III antibodies were demonstrated. Among these is a 1.1 Kb

EcoRI restriction fragment. This fragment was inserted into ompA vectors in all three reading frames (Figure 5). Cells were grown at 37°C in L broth containing 100mg/ml. ampicillin to an ob600 of 0.2. At this time, the cell cultures were divided into two aliquots. IPTG was added to one aliquot to a final concentration of 2 mM (induced). ITPG was not added to the other aliquot (uninduced). Upon ITPG induction, transformants of all three plasmid constructs (designated OmpA<sub>1</sub>-R-6, (O1R6) OmpA<sub>2</sub>-R-7 (O2R7), and OmpA<sub>3</sub>-R-3 (O3R3)) produced a 15 Kd peptide that is strongly reactive with anti-HTLV-III antibodies in sera from AIDS patients (Figure 6 lane 1, purified HTLV-III virions; lanes 2 and 3, O1R6 uninduced and induced; lanes 4 and 5, O2R7 uninduced and induced; lanes 6 and 7 O3R3 uninduced and induced). This reactivity is not detected when sera from normal individuals is used.

DNA sequence data of the HTLV-II genome indicates that there is an open reading frame inside the pol gene located at the 5'-end of the EcoRI fragment. DNA sequence analysis of the three recombinant constructs, O1R6, O2R7 and P3R3, confirmed that each of these recombinants has a different reading frame of the HTLV-III plus strand coupled to the coding sequence of each vector. Only in O3R3 is the reading frame of the inserted DNA in phase with that set by the signal peptide in the ompA vector, in O1R6 and O2R7 the pol gene segment DNA is out of phase (Figure 6a),

There is a 6 bp ribosome binding site, AAGGAG (Shine-Dalgarno sequence), located at nucleotide position 24-29 and an initiation codon, ATG, located 11 bp downstream (position 41-43). The 15 Kd peptide synthesized by all three recombinants appears to be translated from the transcript using this internal codon. If this is true, the peptide starts from the ATG located at position 41-43 and ends at the stop codon at position 446-448, producing a peptide of 135 amino acid residue encoded by the 3'-end segment of the pol gene of HTLV-III.

In addition to the 15 Kd peptide, the O3R3 construct, in which the reading frame of the HTLV-III DNA pol gene is in phase with that set by the vector, produced two additional peptides about 19 Kd and 16.5 Kd in size (Figure 6). It is possible that the 19 Kd peptide contains an additional 35 amino acid residues, 21 of which are from the signal peptide encoded by the ompA<sub>3</sub> vector and 14 encoded by the inserted HTLV-III DNA itself. The 16.5 Kd peptide may be the processed 19 Kd peptide in which the signal peptide is cleaved.

The O1R6 and O2R7 constructs also produces another peptide of about 17.5 Kd (Figure 6) and weakly reactive with sera of AIDS patients. The origin of this peptide is not clear. The 1.1 Kb EcoRI fragment contains a second potential coding region designated as the short open reading frame (SOR) extending

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from nucleotide position 260 to 965 (Figure 5). Four of the five AUG methionine codons in this region are near the 5'-end of this open reading frame. This DNA segment could encode peptides of 192, 185, 177 or 164 amino acid residues. However, there is no clearly recognizable ribosome binding site at the 5'-end of this open reading frame.

Further evidence also supports the conclusion that the 15 Kd peptide is indeed derived from the pol gene. First, deletion of the 3'-end Stul to EcoRI fragment from the 1.1 Kb EcoRI insert from O1r6, O2R7 and O3R8 (Figure 5) does not affect the synthesis of the 15 Kd peptide. Second, clones containing only the 5'-end EcoRI to Ndel fragment still produce the same 15 Kd peptide. Finally, several recombinant clones containing various DNA fragments having the SOR coding sequence properly inserted into the open reading frame cloning vector, pMR100, produced lambdaCI-HTLV-III B-galactosidase tripartite fusion proteins which have very little immunoreactivity with anti-HTLV-III antibodies present in sera from AIDS patients.

Significant immunoreactivity against the 15 Kd peptide derived from the viral pol gene in sera from AIDS patients was detected. The identify of this immunoreactive peptide, with respect to the banding pattern of HTLV-III virion antigen in SDS-polyacrylamide gel electrophoresis, was determined by means of a competition inhibition immunoassay. Purified HTLV-III virions were treated with SDS, electrophoresis, and electroblotted onto a nitrocellulose filter. Identical filter strips containing disrupted HTLV-III virions were incubated with well characterized serum from an AIDS patient in the presence or absence of lysates of O1R6, O2R7, or control bacterial clones. The specific immunoreaction between anti-HTLV-III antibodies present in sera of the AIDS patients and the blotted virion proteins were then revealed by 1251labeled goat anti-human antibody. As shown in Figure 7, lysates of O1R6 block the immunoreactivity of the viral p31 protein with the AIDS serum, while lysates of control cells do not. This result suggest that the recombinant 15 Kd peptide encoded by 3'-end of the viral pol gene is also a part of another virion protein, p31, in contrast to the view shared by some that p31 is a cellular protein which co-purifies with HTLV-III virions.

The prevalence in the sera of AIDS patients of antibodies against the 15 Kd peptide was also evaluated. In Western blot analysis employing the lysate of O1R6 as the source of antigen, a panel of coded sera from AIDS patients and normal healthy individuals was tested. All of the 20 AIDS sera and none of the 8 norral control reacted with the 15 Kd peptide. Representative results are shown in (Figure 8). These data indicate that most, if not all, AIDS patents produce antibodies against the viral p31 protein.

#### **EXAMPLE 6**

# EXPRESSION IN E. COLI OF OPEN READING FRAME GENE SEGMENTS OF HTLV-III

HTLV-III DNA was excised from lambda BH-10, which is previously construed recombinant lambda phage containing a 9 Kb segment of HTLV-III DNA inserted into the vector lambdagtwes lambda B (Figure 2a). This HTLV-III DNA was sonicated and DNA fragments of about 0.5 Kb purified by gel electrophoresis, end repaired, and inserted into the Smal site of the open reading frame (ORF) vector, pMR100 (Figure 9). This vector contains a bacterial lac promotor DNA segment linked to a second DNA fragment containing a hybrid coding sequence in which the N-terminus (5' segment) of the lambda CI gene of bacteriophage lambda is fused to an N-terminal-deleted lacIZ gene (3'-segment). A short linker DNA fragment containing a Smal cloning site, has been inserted between these two fragments in such a manner that a frame shift mutation has been introduced upstream of the lacIZ-coding DNA. As a result, pMR100 does not produce any detectable B-galactosidase activity when introduced into cells of the Lac host E. coli LG90. The insertion of foreign DNA containing an open reading frame, in this case the HTLV-III DNA, at the Smal cloning site can reverse the frame shift mutation if the inserted coding sequence is in the correct reading frame with respect to both the lambdaCl leader and the LacIZ gene. Transformants were screened on MacConkey plates to detect individual clones that expressed Bgalactosidase enzymatic activity in situ.

Among the 6000 ampicillin resistant transformants screened, about 300 were found to express B-galactosidase activity. Colony hybridization using <sup>32</sup>p-labelled nick-translated HTLV-III DNA as a probe revealed that all these Lac<sup>+</sup> clones contained HTLV-III DNA. In the Lac<sup>+</sup> clones the HTLV-III fragment inserted into the Sma I site of pMR100 must contain no stop codons in the reading frame set by the lambdaCl leader segment and the lacIZ gene must also be in the correct translational reading frame. The three-element-fused genes were expressed as tripartite fusion proteins, having a portion of the lambdaCl protein at the N-terminus, the HTLV-III segment in the middle, and the lacIZ polypeptide at the C-terminus.

THe proteins produced by the Lac<sup>+</sup> clones were analyzed by resolving cell lysates on 7.5% SDS-polyacrylamide gels along with those of the control Lac<sup>+</sup> clone pMR200, which produced a lambdaCl-B-galactosidase fusion protein. The lacIZ gene in pMR200 is identical to that in pMR100 except that it has a single base pair deletion which brings it in phase with the lambdaCl gene to produce an active B-galactosidase. By virtue of the very large size of the B-galactosidase and its fusion proteins, they are separated from the bulk of proteins in the cell lysates on the SDS-polya-

crylamide gels and can be easily identified by Coomassie brilliant blue staining as shown in Figure 10a. Some of the Lac<sup>+</sup> clones containing HTLV-III DNA produce polypeptides that are larger (15,000 to 27,000 dalton) than the lambdaCI-lacIZ fusion protein. The findings are consistent with data that the DNA inserts are up to 700 bp long. The B-galactosidase fusion proteins accounted for about 1-2% of total cellular protein.

The peptides produced by the Lac+ clones were examined by Western blot analysis for immunoreactivity with sera from AIDS patients. After the lysates of Lac+ clones were electrophoresed in SDS-polyacrylamide gels, they were electro-transferred to nitrocellulose filters. These protein blots were first reacted with AIDS patient sera and then with 1251-labeled goat anti-human IgG. The autoradiograph in Figure 10b shows the immunoreactivity of a representative fused protein with the serum from an AIDS patient. The recombinant peptides also reacted with anti-Bgalactosidase antiserum, consistent with the proposition that they had the general structure lambdaCl-HTLV-III peptide-LacIZ. From the immunoreactivity pattern of the negative controls, pMR100 and pMR200, which do not contain an HTLV-III DNA insert, it is evident that this particular AIDS serum contains antibodies reactive with several bacterial proteins of the host E. coli. This is not surprising, since AIDS patients are usually infected with a number of bacteria. Absorbing AIDS patient sera with Sepharose 4B conjugated with E. coli extract reduced the background immunoreactivity to some extend but did not completely eliminate it.

About 300 independent HTLV-III DNA-containing Lac+ colonies were analyzed in SDS polyacrylamide gels using Coomassie brilliant blue staining and Westerns blotting. About half of them were found to express fusion proteins containing extra peptides of about 100-200 amino acids, corresponding to DNA inserts of 300-600 bp long. Of these fusion proteins, 20 were found to react specifically with sera from AIDS patients. The unreactive clones probably contain peptides that fold in such a way that they are not reactive with antibodies or correspond to regions of HTLV-III protein molecules which are not immunogenic in AIDS patients. The other half of the Lac+ clones expressed fusion proteins whose sizes were not obviously different from that of the lambdaCl B-galactosidase protein. None from this group of fusion proteins was found to react with sera from AIDS patients.

THe HTLV-III DNA inserts from Lac<sup>+</sup> ORF clones were mapped to specific segments in the HTLV-III genome using Southern blotting procedures. In these studies, each plasmid clone was labelled with <sup>32</sup>p by nick-translation and hydribized to a battery of HTLV-III DNA restriction fragments. This hybridization analysis mapped all of the Lac<sup>+</sup> ORF clones into four open reading frame segments designated ORF-A, ORF-B,

ORF-C, and ORF-D (Figure 2a) consistent with the DNA sequencing data. The open reading frames ORF-A and -B, corresponding to the coding regions of the gag and pol genes, are 1.5 Kb and 3.0 Kb long, respectively. ORF-C is about 0.6 Kb long, slightly overlaps with the ORF-B region, and is capable of encoding a polypeptide of 21 overlaps with the ORF-B region, and is capable of encoding a polypeptide of 21 Kd. The location of ORF-C and its overlap with the pol gene are reminiscent of the structure of the env genes in HTLV-I and -II. However, ORF-C, designates as the short open reading frame (sor), is too short to code for the entire envelope protein. The fourth open reading frame, ORF-D is 2.5 Kb long and could encode both a large precursor of the major envelope glycoprotein and another protein derived from the 3' terminus, which may be analogous to the lor products of HTLV-I and -II. this gene region of HTLV-III, designated env-lor, is at least twice as long as the lor of HTLV-I and HTLV-II and it is presently unclear whether single or multiple proteins are encoded here-

Both Southern blotting and DNA sequencing studies were employed to analyze a number of clones. As shown in Figure 2b, the Lac<sup>+</sup> ORF clones expressing fusion proteins immunoreactive with sera from AIDS patients were located in ORF-A (e.g. #175 and #191), ORF-B (e.g. #13, 31, and 162), or ORF-D (e.g. #113, 121, and 127) and not in the sor region. Not all peptides in these regions were immunoreactive, e.g. ORF clone #76 located in ORF-D.

Analysis of the open reading frame structures in HTLV-III posed questions as to which open reading frames(s) corresponds to the env gene. It is possible that the env-lor region in HTLV-III contains all or a part of the env gene in addition to the presumed lor gene. Recent evidence suggests that the lor in HTLV-I encodes a 42 Kd protein involved in the process of viral activation and transformation. When the lysate of one of the ORF clones (#127 in Figure 2b) was tested against sera from 20 AIDS patients and 12 healthy normals in a strip radioimmunoassay based on the Western blot technique, immunoreactivity against the lambdaCI-HTLV-III-B-galactasidase fusion polypeptide was detected in the sera from 19 of the AIDS patients and none from normal controls. This result indicates that the protein encoded by the portion of the env-lor region contained in ORF clone # 127 is produced in HTLV-III infected cells and induces antibody production in most if no all AIDS patients.

#### Industrial Applicability

This invention has industrial applicability in screening for the presence of HTLV-III DNA in body fluids and the diagnosis of AIDS.

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#### Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more that routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

The invention therefore provides an immunoreactive HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III cDNA. The HTLV-III cDNA may encode an env gene sequence, an env-lor gene sequence or be an EcoRI restriction fragment, and/or be immunoreactive with sera of patients with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III.

The invention also provides; (a) isolated HTLV-III envelope polypeptide; or (b) isolated HTLV-III polypeptide encoded by an env-lor gene sequence; or (c) isolated cDNA encoding an HTLV-III gene, which may encode the HTLV-III env gene, or the HTLV-III env-lor gene sequence. The cDNA may also encode an EcoRI restriction fragment of HTLV-III cDNA which encodes a polypeptide immunoreactive with sera of patients with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III, or an HTLV-III polypeptide which is immunoreactive.

Preferably, the isolated cDNA encodes an envelope polypeptide which is immunoreactive with sera of patients with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III, and may be an EcoRI restriction fragment.

The invention also provides a DNA probe comprising a DNA sequence which is essentially homologous to a portion of the HTLV-III genome unique to the virus. The probe may comprise a DNA sequence which is essentially homologous to a portion of the HTLV-III genome which encodes a polypeptide immunoreactive with sera of AIDS patients.

Also covered by the invention is a hybrid protein comprising an HTLV-III polypeptide linked to at least one other polypeptide, e.g. an indicator polypeptide which may comprise beta-galacto-sidase, and an isolated RNA transcript of the <a href="mailto:env">env</a> gene of HTLV-III, which preferably has a label which emits a detectable signal, e.g. a radioisotope.

The invention also provides a recombinant vector containing HTLV-III DNA capable of expression upon insertion into host cells; or an OmpA vector containing HTLV-III cDNA; or a pMR 100 vector containing HTLV-III cDNA.

The invention also provides a method of producing HTLV-III polypeptide, comprising the steps of:

- (a) cleaving HTLv-III cDNA to produce cDNA fragments;
- (b) inserting the cDNA fragments into an expression vector to form a recombinant vector,
- (c) transforming an appropriate host cell with the

recombinant vector; and

- (d) culturing the transformed host cell under conditions sufficient for expression of the polypeptide encoded for by the inserted HTLV-III cDNA. Preferably, the cleaving step comprises:
  - (a) digesting the HTLV-III cDNA with restriction endonucleases to produce restriction fragments of cDNA, or
  - (b) shearing the HTLV-III cDNA to produce cDNA fragments.

Also provided is a method of producing HTLV-III envelope polypeptide, comprising the steps of:

- (a) cleaving HTLV-III genomic cDNA with the restriction endonuclease Sstl:
- (b) digesting the cleaved cDNA with restriction endonucleases sufficient to generate restriction fragments which encompass at least a portion of the env gene;
- (c) isolating the restriction fragments;
- (d) producing DNA fragments of about 200-500 base pairs in length from the restriction fragments;
- (e) isolating the DNA fragments of about 200-500 base pairs;
- (f) inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising an <u>env</u> gene product and beta-galactosidase;
- (g) transforming lac z<sup>-</sup>E.coli cells with the vector; (h) plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a red color;
- (i) culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein;
- (j) obtaining cellular protein from the cultured transformed cells;
- (k) separating the cellular protein obtained;
- (I) contacting the separated protein with sera from AIDS patients to identify protein which is immunoreactive with the sera;
  - (m) isolating the immunoreactive protein; and
  - (n) optionally separating the env gene expression product from the remainder of the hybrid protein.

The invention also provides an antibody (e.g. monoclonal antibody) which is;

- (a) specifically reactive with HTLV-III envelope polypeptide; or
- (b) specifically reactive with HTLV-III polypeptide produced by recombinant DNA techniques.

Also provided is an immunoassay for the detection of HTLV-III employing antibody (e.g. monoclonal antibody)

- (a) which reacts specifically with HTLV-III polypeptide produced by recombinant DNA techniques; or which
- (b) reacts specifically with HTLV-III envelope

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polypeptide.

The invention also provides a sandwich type immunoradiometric assay for the detection of HTLV-III employing an immobilized antibody which reacts with HTLV-III polypeptide and a soluble antibody which reacts with HTLV-III polypeptide, and an assay kit comprising an antibody which reacts specifically with HTLV-III polypeptide bound to a solid phase and a labeled soluble antibody which reacts specifically with HTLV-III polypeptide.

The invention also provides a method of detecting antibodies against HTLV-III in a bodily fluid comprising the steps of:

- (a) contacting an immunoadsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid until antibodies against HTLV-III polypeptide in the bodily fluid bind the solid phase polypeptide;
- (b) separating the immunoadsorbent from the bodily fluid;
- (c) contacting the immunoadsorbent with a labeled HTLV-III polypeptide or labeled antibody against human immunoglobulin; and
- (d) determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.

Also provided is a kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:

- (a) an immunoadsorbent comprising a HTLV-III polypeptide bound to a solid phase; and
- (b) labeled HTLV-III polypeptide or a labeled antibody against human immunoglobulin.

The invention also provides a method of detecting HTLV-III nucleic acid in a bodily fluid (e.g. a cell lysate) comprising the steps of:

- (a) adsorbing the nucleic acid in a bodily fluid onto an adsorbent;
- (b) denaturing the adsorbed nucleic acid;
- (c) contacting the adsorbed nucleic acid with an HTLV-III DNA or RNA probe; and
- (d) determining if the probe hybridizes with the adsorbed nucleic acid, and a hybridoma cell line which produces antibody specifically reactive with HTLV-III envelope polypeptide.

#### Claims

- HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing DNA encoding a gag, env, env-lor sor and/or pol polypeptide, or an immunologically functional equivalent thereof, the polypeptide being immunoreactive with HTLV-III-specific antibody.
- Polypeptide according to claim 1, wherein the DNA; (a) is a fragment of approximately 200-500

base pairs, and/or (b) is a restriction fragment produced by digestion at any of the sites shown in Figs 1 and 2, and/or (c) has the sequence shown in Fig. 3.

- 3. Polypeptide according to claim 1 or claim 2, wherein (a) the DNA encodes an env polypeptide and the DNA sequence extends from approximately nucleotide 5780 to approximately nucleotide 8370 of the HTLV-III DNA as shown in Fig. 3, or a fragment thereof; or (b) the DNA encodes a gag polypeptide (p17) and the DNA sequence extends from approximately nucleotide 335 to approximately nucleotide 730 of the HTLV-III DNA as shown in Fig. 3, or a fragment thereof; or (c) the DNA encodes a gag polypeptide (p24) and the DNA sequence extends from approximately nucleotide 730 to approximately nucleotide 1630 of the HTLV-III DNA as shown in Fig. 3, or a fragment thereof.
- 4. An immunoassay for the detection of antibodies to HTLV-III, comprising the steps of contacting polypeptide according to any one of the preceding claims with a test sample, and then determining the presence of antibody bound thereto.
- Án isolated DNA (e.g. a recombinant vector) comprising a DNA sequence encoding polypeptide according to any one of claims 1 to 3; or a host cell transformed with said DNA.
- 6. A method of producing HTLV-III polypeptide, comprising the step of culturing the host cell of claim 5 under conditions sufficient for expression of the polypeptide.
- 7. A method according to claim 6, wherein the HTLV-III polypeptide is an envelope polypeptide and the HTLV-III DNA is cleaved with restriction endonucleases to generate fragments encompassing at least part of the env gene, comprising the steps of;
  - (a) isolating the restriction fragments,
  - (b) producing and then isolating fragments of about 200 to 500 base pairs in length from the restriction fragments,
  - (c) inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising env gene product and betagalactosidase,
  - (d) transforming lac  $Z^ \underline{E}$ . coli cells with the vector,
  - (e) plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a

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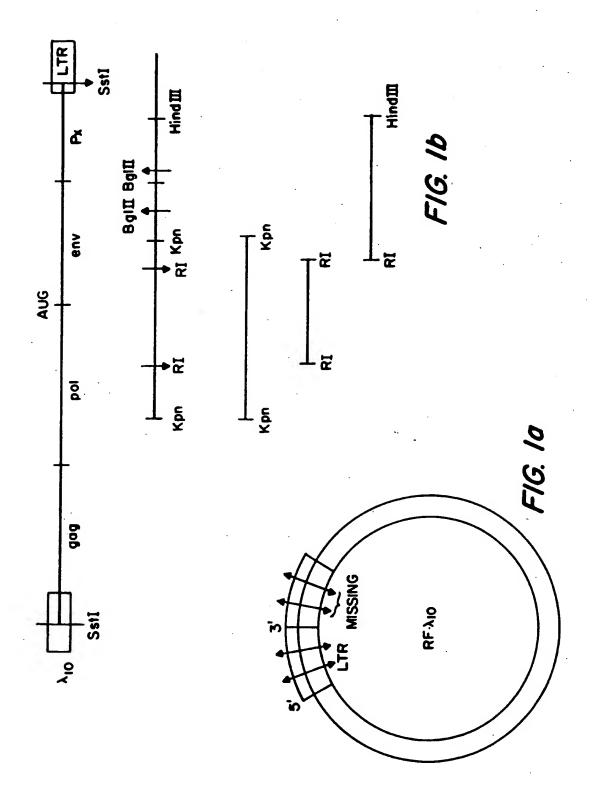
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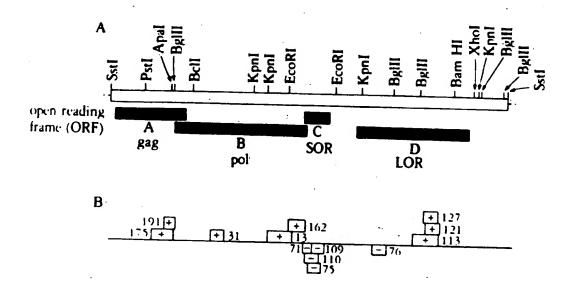
red colour,

- (f) culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein,
- (g) obtaining cellular protein from the cultured transformed cells.
- (h) separating the cellular protein obtained,
- (i) identifying protein which is immunoreactive with the sera,
- (j) isolating the immunoreactive protein, and optionally
- (k) separating the <u>env</u> gene expression product from the remainder of the hybrid protein.
- A hybrid protein comprising polypeptide according to any one of claims 1 to 3, linked to a polypeptide that is not immunoreactive with antibodies to HTLV-III.
- A DNA probe or an isolated RNA transcript comprising a nucleic acid sequence essentially homologous to the DNA as defined in claim 5.
- 10. Monoclonal antibody specifically reactive with the polypeptide according to any one of claims 1 to 3; or a hybridoma cell line which produces said antibody.
- An immunoassay for the detection of HTLV-III employing antibody according to claim 10.
- 12. An assay kit comprising a solid phase bound antibody which reacts specifically with HTLV-III polypeptide and a labeled soluble antibody which reacts specifically with HTLV-III polypeptide, wherein the polypeptide is according to any one of claims 1 to 3.
- 13. A method of detecting antibodies against HTLV-III in a bodily fluid, comprising the steps of:
  - (a) contacting an immunoadsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid until antibodies against HTLV-III polypeptide in the bodily fluid bind the solid phase polypeptide, wherein said polypeptide is according to any one of claims 1 to 3,
  - (b) separating the immunoadsorbent from the bodily fluid,
  - (c) contacting the immunoadsorbent with the labeled HTLV-III polypeptide or labeled antibody against human immunoglobulin, and
  - (d) determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.
- 14. An assay kit for determining the presence of antibody against HTLV-III in a bodily fluid, compris-

ing;

- (a) an immunoadsorbent comprising a HTLV-III polypeptide bound to a solid phase, wherein said polypeptide is according to any one of claims 1 to 3, and (b) a labeled HTLV-III polypeptide or a labeled antibody against human immunoglobulin.
- A method of detecting HTLV-III nucleic acid in a bodily fluid (e.g. a cell lysate) comprising the steps of;
  - (a) adsorbing the nucleic acid in a bodily fluid onto an adsorbent,
  - (b) denaturing the adsorbed nucleic acid,
  - (c) contacting the adsorbed nucleic acid with HTLV-III DNA or RNA probe according to claim 9, and
  - (d) determining if the probe hybridizes with the adsorbed nucleic acid.
- 16. A diagnostic reagent comprising a polypeptide according to any one of claims 1 to 3, which is for example labeled and e.g. further comprises a solid phase on which the polypeptide is immobilized.
- A polypeptide according to any one of claims 1 to 3, for use in therapy, e.g. vaccination.
- 18. Use of a monoclonal antibody according to claim 10 for the manufacture of a medicament for use in immunotherapy against AIDS.
- 19. Use of a polypeptide according to any one of claims 1 to 3 for the manufacture of a medicament for use in vaccination against AIDS.





F16.2

CLONE	NVCLE POS	34170	AMING ACIB RESIDUE
3H10	TOGAAGGOCTAATTCACTCCCAACGAAGACAAGA	-428	
3H 10	TATCCTTGATCTOTGOATCTACCACACACACACGCTACTTCCCTGATTACCACACACA	-345	
BHS			
BH 16 BHS	CAGATATCCACTOACCTTTOGATOGTOCTACAAOCTAOTACCAOTTOAOCCAGAOAAOTTAGAAGAAOCCAACAA	-270	
BH 10 BHS	AGGAGAGAACACCAGCTTGTTACACCCTGTBAGCCTGCATGGAATGGA	-195	,
BH 10 BH8	GAGGTTTGACAGCCCCCTAGCATTTCATCACATGCCCCGAGAGCTCCCGCGAGTACTTCAAGAACTCCTGACA	-120	
3H 10 3H2	TCGAGCTTOCTACAAOGGACTTTCCGCTGOGGACTTTCCAYOGAGOCGTGOCCTGGOCGGGACTGGGGAGTGGCG	-45	
8H10 BH8	TATA BOX PVW II  AGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTACT	-1	
3H 10 3H8	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTC	39	
HXBZ	MING III R TOTOCTAACTAGOOAACCCACTOCTTAAOCCTCAA	75	
NXBZ	TAAR CTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGTG	150	
HXBZ	CCCTTTTAGTCAGTGTGGAAAATCTCYAGCAGTGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCA	221	
BH 10 BH5	GAGCTCTCTCGACGCAOGACTCGGCTGACAGCGCCCACGGCAAGAGGCGAGGGCCGGCGACTGGTGAGTACG	296	
BH 10 BH5	Leader sequence —— GAG #17 CCAAAAATTTTGACTAGCGGGGGCTAGAAGGAGAGAGAGA	57 1	13
BH 10 BHS	AGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAA	446	38
BH 10 BH 5	CAGGOAGCTAGAACGATTCOCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGOCTGTAGACAAATACTGGGACA ArgGluLeuGluArqPheAlaYalAsnProGlyLeuLeuGluThrSerGluGlyCysArgGlnIleLeuGlyGln	521	63
3H10 3H5	GCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGT LauGlapproSeplauGiaThpGlySepGluGlulauArgSeplauTypAanThrValAlaThrLauTypCysVal	596	44
BH 18 BH5	Wind III GCATCANAGGATAGAGATAAAAGACACCAAGUAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAA	 671	113
BH 10 BH5	Puu II GAG p17 —— GAG p24 AAAAGCACAGCAAGCAGCTGACACAGGACACAGGACATCAGCCAAAATTACCCTATAGTOCAGAACAT LysalisGinginalsalsalsabpThrGlyHisSerSerGlnValSerGlnAunTyPProlleValDinAunlie	746	138
3H10 3H5	CCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAAGGAAG	821	163
BH 10 BH 5	TTTCAGCCCAGAAGTAATACCCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAGAGTAAAACACCATOCT PheSerProGluVallleProMetPheSerAlaLeuSerGluGlyAlaThrProGlnAspLeuAenThrMetLeu	896	188
BH 10 BH 5	AAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGA AanThrvaldiyGlyHiaGlaAlaAlaMetGlaMetbulyaGluThrllaAanGluGluAlaAlaGluTrpAap	971	213
BH 10 BH5	TAGAGTACATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGG ArqValNisProValNisAlaGlyProllaAlaProGlyGlaMatArqGluProArqGlySarAspllaAlaGly	1846	238
BH 10 BH 5	AACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTAGGAGAAATTTATAA ThrThrSerThrLeuGlnGluGlnlleGlyTrpMetThrAshAbnProProIleProValOlyGluIleTyrLys	1121	263
BH 10	AAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAATGTATAOCCCTACCAGCATTCTGGACATAAGACAAGO ArgTrpIleIleLeuGlyLeuAenLyeIleVelArgMetTyrSerProThrSerIleLeuAepIleArgOlnGly	1196	288

<b>B</b> H10	Mind 111 ACCAAAAGAACCTTTTAGAGACTATOTAGACCOOTTCTATAAAACTCTAAGAOCCGAGCAGCTTCACAGGAGGT Prolymulufraphaargaaptyrvalaapargphatyrlysthrlauargalagudaalasargingluval	127 1	313
BH5	Ahe 111		• • •
3H10 3H5	AAAAAATTOGATGACAGAACCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATTGGG LysAsnTrpMetThrGjuThrloulouVolGjnAsnAjaAsnProAspCysLysThrIjeLouLysAjaLouGjy	1346	331
3H10 BH5	ACCAGCOCTACACTAGAAGAATGATGACAGCATGTCAGGGAGTAGGAGGACCCGGCCATAAGGCAAGAGTTTT ProAlbAlbThrleuGluGluMetMetThrAlbCyaGlnGlyValGlyGlyProGlyHlalyaAlbArgVallau	1421	363
BH16 BH5	GGCTGAAGCAATGAGCCAAGTAACAAATACAGCTACCATAATGATGCAGAGAGGCAATTTTAGGAACCAAAGAAA AlaGluAlaMetSarGinValThrAanThrAlaThrIlaMetMetGinArgGiyAanPhaArgAanGinArgLya T-A	1496	388
BH 10	GATOGTTAAGTOTTTCAATTGTGGCAAAGAAGGGCACACAGCCADAAATTGCAGGGCCCCTAGGAAAAAGGGCTG MetVallysCysPheAsnCysGlyLysGlyGlyHisThrAlsArgAsnCysArgAlsProArgLysLysGlyCys A-T	157 1	413
BH 10 BH5	TTGGAAATGTGGAAAGGAAGGACACCAAATGAAAGATTGTACTGAGAGACAGGCTAATTTTTAGGAAAGATCTG TPPLYSCYSGIYLYSGIUGIYHISGInMettysAspCysThrGluArqGlnAlsAsnPhelauGiytysIleTrp PhePheArqGluAspleu	1646 -	438
BH 18 BH 5	GCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCATTTCTTCAGAG ProSerTyrlysGlyArgProGlyAsnPheleuGlnSerArgProGluProThrAlaProProPheleuGlnSer AlaPheleuGlnGlyLysAlaArgGluPheSerSerGluGlnThrArgAlaAsnSerProThrIleSerSerGlu	1721	463 31
BH 16	Repeat  CAGACCAGAGCCAACAGCCCCACCAGAAGAAGACTTCAGGTCTGGGGTAGAGACAACAACTCCCCCTCAGAAGCA  ArgProGluProThrAlaProProGluGluSerPhaArgSerGlyValGluThrThrThrProProGlnLysGln GlnThrArgAlaAsnSerProThrArgArgGluLeuGlnValTrpGlyArgAspAsnAsnSerProSerGluAle	1796	48 8 56
BH5	Ser Leu GAG p151	1871	
BH 10	GGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTCTTTGGCAACGACCCCTCGTCACAATA GluProIleAsplysGluLeuTyrProLeuThrSerLeuArgSerLeuPheGlyAsnAspProSerSerGin GlyAlaAspArgGinGlyThrYalSerPheAsnPheProGinIleThrLeuTrpGinArgProLeuValThrIle	,	512 81
BH5 BH10 BH5	AAGATAGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGADCAGATGATACAGTATTAGAAGAAATGAGTTTG LysIleGlyGlyGlnteulysGluAlaleuleuAspThrGlyAlaAspAspThrValLeuGluGluHetSerleu	1946	106
BH10 BH5	CCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTC ProGlyArqTrplysProlysMetileGlyGlyIleGlyGlyPheIleLysValArqGlaTyrAspGlaIleLeu	202.1	131
BH 10 BH 5	ATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAAT IleGluIleCysGlyHisLysAlaIleGlyThrValLeuValGlyProThrProValAsnIleIleGlyArqAsn Aha III	2096	156
BH10 BH5	CTGTTGACTCAGATTGGTTGCACTTTAAATTTTCCCATTAGCCCTATTGAGACTGTACCAGTAAAATTAAAGCCA LeuleuThrGlnIleGlyCysThrleuAsnPheProIleSerProIleGluThrValProVallysLeulysPro	2171	18 1
BH 10 BH 5	GGAATGGCCCAAAAGTTAAACAATGGCCATTGACAGAAAAAAATAAAAGCATTAGTAGAAATTTGTACA GlyMetAspGlyProlysVallysGinTrpProleuThrGluGluLysIIeLysAlaLeuValGluIIeCysThr	2246	206
3H10 3H5	GAAATGGAAAGGAAGGGARAATTTCAAAAATTGGGCCTGAGAATCCATACAATACTCCAGTATTTGCCATAAAG GluNetGlulysGluGlyLyslleSerLyslleGlyProGluAsnProTyrAsnThrProValPheAlaIleLys	2321	231
BH 10 BH5	AAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGACTCAAGACTTCTGGGAA LysLysAspSerThrLysTrpArqLysLeuValAspPheArqGluLeuAsnLysArqThrGlnAspPheTrpGlu G	2396	256
BH 10 BH 5	GTTCAATTAGGAATACCACATCCCGCAGGGTTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTGATGCA ValGintauGlyliaProhiaProhiaGlylautysLysLysLysSerValThrValtauAapValGlyAapAla	247 1	28 1
BH 10 BH 5	TATTTTTCAGTTCCCTTAGATGAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACA TyrPheSerValProLeuAspGluAspPheArqLysTyrThrAlaPheThrIleProSerlleAsnAsnGluThr	2546	304
BH10 BH5	CCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATG ProGlylleArqTyrGlnTyrAsnVolLeuProGlnGlyTrpLysGlySerProAlaIlaPheGlnSerSerMet	2621	331
BH 10 BH5	SerGly  Aha III  ACAAAAATCTTAGAGCCTTTTAAAAAACAAAATCCAGACATAGTTATCTATC	2496	356
	<b></b>		

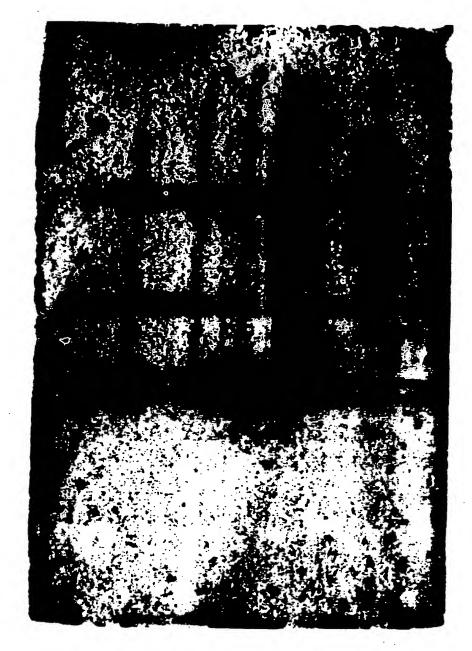
BN 18 BH 5	OATCTOACTTAGAAATAGOGCAGCATAGAACAAAATAGAGGAGCTGAGACAACATCTGTTGAGGTGGGGGACTT OlyserAsployGlullgGlyGlnHsArqTrrlygllgGlyGluLauArqGlnHslauLauArqTrgGlyLau Pho	2771	34
BH 18 BH5	ACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGA	2844	400
BN 10 BN 5	ACAGTACAGCCTATAGTGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTAGTGGGGAAATTU ThrvalGinProllevalLouProGluLyaAspSerTrpThrvalAsnAspIloGlnLyaLouValGlyLyaLou Ilo	2921	43
BH 10 BH 5	AATTOOOCAAGTCAGATTTACCCAGOGATTAAAGTAAGGCAATTATGTAAACTCCTTAGAGGAACCAAAGCACTA Aantraalasardinilatypproglyllalysvalargdinlaucyalyslaulauargdlythelysälalau	2996	456
BH 10 BH 5	ACAGAAGTAATACCACTAACAGAAGAAGCAGAGCTAGAACTOGCAGAAAACAGAGAGATTCTAAAAGAACCAGTA ThrGluYallleProtouThrGluGluAlaGlutouUlutouAlaGluAsaArgGlulletoutysGluProYal	3071	481
BH 10 BH5	CATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAOCAGGGGGCCAATGGACATAT NigGlyVolTyrTyrAspProSerlysAspleulleAlaGlulleGlnlysGlnGlyGlnGlyGlnTrpThrTyr	3146	500
BH 18 BHS	Aha III CAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAATATGCAAGAATGAGGGGTGCCCACACTAATGAT GInIleTyrOlnOluProPhelysAsnleulysThrOlyLysTyrAlsArgMetArgGlyAlsHisThrAsnAsp	3221	531
BH 18 BH 5	Aha GTAAAACAATTAACAGAGGCAGTGCAAAAAATAACCACAGAAACCATAGTAATATOOGGAAAGACTCCTAAATTT VallysGinlauThrOluAlsValGinlysIlsThrThrGluSerIlsValIlsTrpGlyLysThrProlysPha	3296	554
BH18 BH5	AAACTACCCATACAAAAGGAAACATGGGAAACATGGTOGACAGAGTATTOCCAACCCACCTGGATTCCTGAGTOG LysLauProllaGlnLysGluThrTrpGluThrTrpTtpTtpGluTyrTrpGlnAlaThrTrpIlaProGluTrp	337 1	58 1
BH18 BH5	GAGTTTOTTAATACCCCTCCTTTAGTGAAATTATÖÖTACCAGTTAGAGAAGAACCCATAGTAGGAGCAGAAACC Gluphavalaanthrproprolouvallyalautrptyrginlouglulyagluprollavalglyalagluthr	3446	694
8H.10 8H5	TTCTATGTAGATGGGGCAGCTAACAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTAACAAAGGAAGACAA PheTyrValAspGlyAlaAlaAshArgGluThrLysLeuGlyLysAlaGlyTyrValThrAshLysGlyArgGln G	3521	631
3H18 3H5	AAGGTTGTCCCCCTAACTAACAACAAATCAGAAAACTGAGTTACAAGCAATTTATCTACCTTTGCACGATTCA LysValValValValValAcaachacaacaaatcagaaaaactgagttaCaagcaatttatctacctttaCacgaattCa LysValValValValValValValValValValValValValV	3594	456
8H 18 BH5	GGATTAGAAOTAAACATAGTAACAGACTCACAATATOCATTAGGAATCATTCAAGCACAACCAGATAAAAGTGAA GlyLeuGluValAenileValThrAepSerGlnTyrAlaLeuGlyIleIleGlnAlaGlnProAsplysSerGlu	3671	681
BH 1 B BH5	TCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTATCTGGCATGGGTACCAGCACAC SerGluleuvalAenGlnileIleGluGlnleuIleLysLysGluLysValTyrLeuAlaTrpValProAlaHis	3746	706
8H18 BHS	AAAQQAATTOQAQQAAATQAACAAQTAQATAAATTAGTCAQTGCTQGAATCAQGAAAATACTATTTTTAQATQQA LysGlyIleGlyOlyAsnGluGlnValAspLysLeuValSerAlaGlyIleArgLysIleLeuPheLeuAspOly	3821	731
BH 1 0 BH 5	ATAGATAAOOCCCAAGATGAACATGAGAAATATCACAGTAATTOGAGAGCAATGOCTAGTGATTTTTAACCTOCCA IlaksplysälsülääspüluHisüluLysTyrHisSeräsnTrpArgAlsHetälsSeräspPheAsnlouPro	34 96	756
BH 1 0 BH 5	Pvu II  CCTOTAOTAOCAAAAOAAATAGTAGCCAOCTGTGATAAATGTCAOCTAAAAGGAGAAOCCATGCATOGACAAGTA ProveivalalelyeGluilevelaleSerCyeAeplysCyeGlnleulyeGlyGluAleMetHieGlyGlnVel	3971	781
BM 1 0 BM 5	OACTOTAGTCCAOGAATATOCCAACTAGATTOTACACATTTAGAACGAAAAGTTATCCTCCTACCAGTTCATGTA AspCysSerProGlylloTrpGlaLouAspCysThrHislouGlyLysVollioLouVolAloVolHisVol	4046	
IN 18 IN 5	OCCADTOGATATATAGAAOCAGAAGTTATTCCAOCAGAAACAOOOCAGGAAACAOCATATITTCTTTAAAATTA AlaSarOlyTyrIlaGluAlaGluValIlaProAlaGluThrOlyGlaGluThrAlaTyrPhalaulaulyalau	4121	831
M 10 MS	GCAGGAAGATOOCCAGTAAAAACAATACATACAGACAATOOCAGCAATTTCACCAGTGCTACGGTTAAGOCCGCC AlaGlyArgTrpProValLysThrIlaHisThrAspAssGlySerAssPheThrSerAlaThrValLysAlaAla Eze RI	4196	154
H10	TGTTGGTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	427 1	881
IN 18	AATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCA	4346	904

3#18 8#3	GTATTCATCCACAATTTTAAAAGAAAAGOOGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATA ValphollemisAsnPholysArqlysGlyGlyTisGlyGlyTyrSarAlsGlyGluArqlisValAspIisIls	4421	93
BH18 BHS	@CAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTCOOOTTTATTACAOGGAC Aleth-AeplieOinTh-LyeOiuleuGinlyeOinIleTh-LyeIleGinAenPheArgVelTyrTyrArgAep	4496	930
BH10 BH5	ACCAGAAATCCACTTTOGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAAT SerArgAenProleuTrplyeGlyProAlalysieuleuTrplyeGlyGlyGlyAlaValValIleGlnAepAen	4571	98
BH 10	AGTOACATAAAAGTAGTOCCAAGAAGAAAAGCAAAGATCATTAGGGATTATGGAAAACAGATGGCAGGTGATGAT SerAepilelysValValPreAreGaculysAlelysIleilsArgAseTyrGlylysGInMetAlsGlyAspAse CysGInGlyGlyLysGInArgSerLeuGlyIleMetGluAsnArgTrpGInValMetIle	4646	100
BH5 BH18	POL —— TOTOTOOCAAOTAOACAOOATOAOAATTAOAACATOGAAAAGTTTAGTAAAACACCATATGTATGTTTCAOOGAA Cyayaiaiaiaserargginaagoluaag ValtrpolnyalaagargymetärgiiaargthrtrplyeSerleuvallyeHiaHiaMetTyrvalSerOlylye	4721	101
B#5	Arg		
8H 10	AQCTAQQQQATQQTTTTATAQACATCACTATGAAAQCCCTCATCCAAQAATAAGTTCAGAAQTACACATCCCACT AleargGlyTrePheTyrArgHisHisTyrGluSerProHisProArglleSerSerGluValHislleProLou	4796	. 70
BH10 BH5	AGOOGATOCTAGATTOGTAATAACAACATATTOGOGTCTOCATACAGGAGAAAGAGACTGOCATTTGGGTCAGGG GlyAspAlsArgLauVslllaTheTheTyrTrpGlyLauHisTheGlyGluArgAspTrpHisLauGlyGlnGly	4871	•!
BH10 BH5	AGTETECATAGAATGGAGGAAAAAGAGATATAGCACACAAGTAGACCETGAACTAGCAGACCAACTAATTCATCT ValSerIleGluTrpArglyslyaArgTyrSerThrGlnValAapProGluLeuAlaAapGlnLeuIleHieleu	4946	120
BH10	Arg  OTATTACTTTOACTGTTTTTCAGACTCTOCTATAAGAAAGOCCTTATTAOGACACATAGTTAGCCCTAGGTGTGA  TyrTyrPhaAsgCysPhaSerAspSerAlsIlaArgLysAlsLauLsuGlyHisIlaYslSerProArgCysGlu	5021	145
BH 10 BH 10 BH 5	ATATCAAQCAQQACATAACAAQQTAQQATCTCTACAATACTTQQCACTAQCAQCATTAATAACACCAAAAAAQAT TyrQlnalaQlyNisAsnlysVelGlySerleuQlnTyrleuAlaleuAlaleuBleThrProlyslysIle	5096	176
BH 18 BHS	AAAGCCACCTTTGCCTAGTGTTACGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACCAAGGCCCAGAAGACCAAGGCCCAGAAGA	5171	199
BH10 BH5 .	SOR ————————————————————————————————————	5246	203
BH 10 BH 5	ATTTOOCTCCATGGCTTAGGGCAACATATCTATGAAACTTATGGGGATACTTGGGCAGGAGTGGAAGCCATAATA	5321	
BH 18 BH5	AĞÄÄTTČTOCAACAACTOCTOTTTATCCATTTTCAGAAT10GOTĞTĞĞACATAQCAGAATAOCGTTACTCGACA	5396	
BH 18 BHS	BAGGAGAGCAAGAAATGGAGCCAGTAGATCCTAGACTAGA	5471	٠
BH18 BH5	CTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTT	5546	
3H 10 3H5 3H8	CCTATOOCAOGAAGAAOCOGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAA	5621	
8H 18 BH8	AGCAGTAAGTAGTACATGTAATGCAACCTATACAAATAGCAATAGTAGCAL 3TTAGTAGTAGCAATAATAATAGCA	A 54	96
3H 10 SK8		5771	
3H10 3H8	CACTAATAGAAGAGCAGTAGCAATGAGAGTGAAGGAGAATATCAGCACTTGTGGAGATGGGGTGG LysGluGlalysThrValAlaMetArgValLysGluLysTyrGlaHisLeuTrpArgTrpGlyTrp	5844	22
3H 10 3H8	ArgTrp01yThrMetteuteu01yMetteuMetIIeCysSerAleThr01utysLeuTrpVeIThrVeITyrTyr	5921	47
<b>D</b> H 10	Pho  Ep. 1  OGGTACCTOTGTGOAAGGAAGCAACCACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAGAGGTA GlyValProValTroLygGluAlaThrThrThrLouPhoCygAlaSerAscAlaLygAlaTyrAcoThrGluVal	5996	72

BH 10 BHS	CATAATGTTTTGGGCCACACATGCCTGTGTACCCACAGAGCCCCAACCCACAGAAGAGTAGTATTGGTAAATGTGACA HighanvaltegalaTheHighlaCysvalProTheAgpProAgnProGleGluValValLguValAgnValThe	4071	91
3K10 3K8	BAAAATTTTAACATOTOGAAAAATGACATBOTAGAACAGATGCATGAGGATATAATCAGTTTATOGGATCAAAGC BluAnnPhoAnnMetTrpLynAnnAnpMetVelBluGlnHetHIsOluAnpIleIleSerLeuTrpAnpOlnSer	6 146	121
3 H 10 3 H 2	Ahe III  CTAAAGCCATGTGTAAAATTAACCCCACTGTGTGTTAGTTTAAAGTGCACTGATTTGAAGAATGATACTAATACC LoulysProCysYallysLouThrProLouCysYalSerLoulysCysThrAspLoulysAsnAspThrAsnThr	6221	147
Вн 10 Вна	AATADTAGCAGCAGGAGAATDATAATGGAGAAAGGAGAGAGATAAAAACTGCTCTTTCAATATCAGCACAAGCATA Amserserserdiyarghelileheldiulysgiydiullelyaamcyaserpheamilesertheserlle	6296	172
BH 18	Lys  ###################################	6371	197
3K19	TATACOTTOACAAOTTOTAACACCTCAOTCATTACACAOOCCTOTCCAAAOOTATCCTTTGAOCCAATTCCCATA TyrThrlauThrSarCysAanThrSarValIlaThrOlnAlaCysProLysValSarPheOluProIlaProIla	6446	222
BHS BH 10 BHZ	CATTATIGTOCCCCOOCTGGTTITOCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACA NistyrCysAlaProAlaGlyPheAlaIlelsuLysCysAsnAsnLysThrPheAsnGlyThrGlyProCysThr	6521	247
BH 10 BH8	# AATGTCAGCACAGTACAATGTACACATGGAATTAGOCCAGTAGTATCAACTCAAC	6596	272
BH10 BH8	OCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAA AlaGluGluGluValVallaArgSarAlaAmphathrAapAsmAlaLyaThrIleIleValOlnLauAsmGla	6671	297
BH18 BH8	Yel AspThr TCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGAGAG	6746	322
BH 10 BH8	Lye Spe  GCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATAAC AlaPheValThrIleGlyLysIleGlyAshRetArgGlaAleHisCysAsnIleSerArgAleLysTrpAshAsn -QC-	6821	347
BH18 BK&	Ala Ata III ACTITAAAACAGATAGATAOCAAATTAAGAGAACAATTTOGAAATAATAAAACAATAATCTTTAAOCAGTCCTCA Thrloulys01nI1sAspSerlysLeuArgGlu0InPheGlyAsnAsmLysThrl1sI1sPheLys0InSerSer	6896	372
9H18	GGAGGGGACCCAGAAATTGTAACCCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACAACTG GlyGlyAspProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCysAsnSerThrOlnLeu	4971	397
BH 1 B BHB	TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAOTGACACAATCACC PhoAssSerThrTrpPhoAssSerThrTrpSerThrLysGlySerAssAssAssThrGluGlySerAspThrlleThr	7846	422
H18	CTCCCATGCAGAATAAAACAAATTATAAACATOTOOCAGGAAGTAOGAAAAQCAATOTATOCCCCTCCCATCAGT LeuProCysArqIleLysGlnIleIleAsnMetTrpGlnGluValGlyLysAlaMetTyrAlsProProIleSer	7121	447
)H 1 8	GOACAAATTAGATOTTCATCAAATATTACAOGGC*GCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCC GlyGlnIleArgCyeSerSerAsnIleThrGlyLeuLeuLeuThrArgAspGlyGlyAenSerAsnAenGluSer	7 196	472
H10	Bgl II GAGATCTTCAGACCTGGAOGAGGAGATATGAGGGACAATTGGAGGAAGTGAATTATAAATATAAAGTAGAAAA GIUIlarhaArgroGlyGlyGlyAspRatArgAspAsnTrpArgSarGlutauTyrLysTyrLysValValLys	7271	497
IN 18 IMB	ATTOMACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAAA	7346	522
H + 8	QQAQCTTTGTTCCTTQQQTTCTTQGGAQCAQCAQCACCATATQQQCQCACQTCAATQACQCTQACQQTACAQ QlyAleLeuPheLeuGlyPheLeuGlyAleAleGlySerThrHetGlyAleAleSerHetThrLeuThrVelQln	7421	547
H19 H8	OCCAGACAATTATTGTCTGOTATAGTGCAGCAGCAGCAGCAATTTOCTGAGGGCTATTGAGGCCCCAACAGCATCTG AlaArgGlnlaulau3arGlyllaValGlnGlnGlnAsnAsnlaulauArgAlaIIaGluAlaGlnGlnHislau Gly	7496	572
H 18	TTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGAAA	<b>75</b> 7 1	597
H 10	CARCITECTOROGATTTOOOCTTGCTCTOOAAAACTCATTTOCACCACTGCTGTGCCTTGOAATGCTAGTTGOAGT	7646	477

Lew 11 eM 1 e 2011 e 20	M 18	### ##################################	7721	647
BM19 TODOCCADITYGTODANTTOGTTTA BM19 TODOCCADITYGTODANTTOGTTTA BM19 TODOCCADITYGTODANTTOGTTTA BM19 TODOCCADITYGTODANTTOGTTTA BM19 TODOCCADITYGTODANTTOGTTTA BM19 TOCTTOGTADOCTTAADAATAGTTTTTCCTOTACTTTCTOTACTTATAAAATTATCATAATAGTTAGTAADAT BM19 TOCTTOGTADOCTTAADAATAGTTTTTCCTOTACTTTCTGTAGTGAATAGAGTTAGGCAGCCAATTACACATTA BM19 BM19 TCOTTTCAGGACCCACCTCCCAATCCCOAOOOGACCCGACCOACCCGAAGGAATAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAG			7796	400
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BM10 TCOTTTCAGACCCACCTCCCAATCCCOOQOQACCCGACAQCCAAQAAATAQAAQAAQAAQAAQAAQAAQAAQAAQAAQAAQA		TrpAlaSeriouTrpAenTrpPheAenIleThrAenTrpLouTrpTyrIleLyclouPheIleMetIleValOly	7871	697
DNIS  TCOTTTCAGACCCACCTCCCCAATCCCOAGOOGACCCGACAGOCCCGACGGAAAGAAAAAAAAA		@lyleuVo1@lyleuArqIleVo1PheAleVo1LeuSerVo1Vo1AenArqVo1Arg@in@lyTyrSerProleu	7946	722
Septhodinin-Histowprolia-roargoly-roaspargh-roaludiy11ediudiudiudiydiydiudra Asan  BH19  GACAGAGACAGATCCATCCATTCCATTACTCAACCGACTTATCCTOCOACCATTCCCCCCCCCC				
Asphrahaphraseriserisergewisianglyseriewalstewistrphasphelewargeriewcystew  Ticaoctaccaccoctigagagactiactcttgattgtaccagoattgtacacagoattgtagagacttctgogaccagoagottop  Phase-Tyrhiearglewargaspleulewiewiintragiievaldulewlewolyargarggiytrp  Bhis Garacctcaaatattgotogaatctcctacagtattggatcagoactaagaactagoatgtagtagatewiew  Bhis Garacctcaaatattgotogaatctcctacagtattggatcagoactaagaactagaatagatagattattatt  Bhis Garaccccaaatactaaatgatagacagoactagatagaagaatttgattagatagatagattyargatsiie  Bhis Aatoccacaactataccagaagaataagacagoactagaagaagaatttgatagatgatagatgatagaagaaga		SorPhoCinThrHislauProIIoProArgGlyProAspArgProGluClyIloGluGluGluGluGlyGlyGluArg	8021	747
Asphrahaphraseriserisergewisianglyseriewalstewistrphasphelewargeriewcystew  Ticaoctaccaccoctigagagactiactcttgattgtaccagoattgtacacagoattgtagagacttctgogaccagoagottop  Phase-Tyrhiearglewargaspleulewiewiintragiievaldulewlewolyargarggiytrp  Bhis Garacctcaaatattgotogaatctcctacagtattggatcagoactaagaactagoatgtagtagatewiew  Bhis Garacctcaaatattgotogaatctcctacagtattggatcagoactaagaactagaatagatagattattatt  Bhis Garaccccaaatactaaatgatagacagoactagatagaagaatttgattagatagatagattyargatsiie  Bhis Aatoccacaactataccagaagaataagacagoactagaagaagaatttgatagatgatagatgatagaagaaga	N 18	GACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTC	2094	
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GARACCECTAGATATTOTOGAATCTCCTACAGTATTOGAGTCAGGAGCTAAAGATAGGACTTAGCTTGCTC  BM8  ATOCCACAGCTATAGCAGTAGCAGTAGCTGAGGGGGGGACAGAGATAGGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGG	-	PheSerTyrHI sArgl ouArgAspl out out out 16ValThrArgI 16ValGlut out ouGlyArgArgGi yTrp	8 17 1	797
AATOCCACAOCTATAOCAGTAGCTGAGGGGACAGATAGGGTTATAGAAGTAGTACAAGGAGCTTATAGAAGTAGTATATAGAAGTAGTATATAGAAGTAGTA		GAAGCCCTCAAATATTGOTGGAATCTCCTACAGTATTGGAGTCAGGAGCTAAAGAATAGTGCTGTTAGCTTGCTC @luAlsloulysTyrTrpTrpAsnloulouGlnTyrTrpSer@ln@luloulysAsnSerAisYalSerloulou	8246	822
Amaistralisississississississississississississi	-	Asn		
DHIS COCCACATACCTAGAAGAATAAGACAGGGCTTGGAAGAATCTTCCTATAAGATGGGCAAGTGGCCAAAAAAG  BMS  TAGTGTGGTTGGATGGCCTGCTGTAAGGGAAAGAATGAGCAGCTGGACCAGCAGCAGCAGCAGCTGGGAGCCAGC  BMS  TAGTGTGGTTGGATGGCCTGCTGTAAGGGAAAGAATGAGCAGCTGAGCCAGCAGCAGCAGCAGCTGGGAGCCAGC  BMS  TAGTGTGGTTGGATGGCCTGCTGTAAGGGAAAGAATGAGCAGCAGCTGAGCCAGCAGCAGCAGCAGCTGGCCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC	_	AmaiaThrAlaIleAlaValAlaGluGlyThrAmpArgValIleGluValValOlnGlyAlaTyrArgAlaIle	8321	847
APPNI BILLO PROPAGA COLLAR CONTROL CON		· ENV-LOR ————		
TOTOGOTAGGCCACAGAGGCCACTOCTTAAGCCCCCTCAGGAGGCCTCACCTCA	-	ArgHislieProArgArgIleArgGinGiyLeuGluArgIleLeuLeu	8396	843
ATCTCGAGACCTAGAAAAACATGGAGCAATCACAAGTAGCAACACAGCAGCAGCTACAATCATGATGTGCTTGGCTTGAGABBHS  BHIS  AGAAGCACAAGAGGAGGAGGAGGAGGTGGTTTCCAGTCACCCCCAGGTACCTTTAAGACCAATGACTTACAAGGC  BHIS  AGAAGCACAAGAGAGAGAGAGGAGGTGGGTTTCCAGTCACCCCCAAGGTACCTTTAAGACCAATGACTACAAGGC  BHIS  AGCTGTAGATCTTAGCCACTTTTAAAAGAAAAAGGGGGGGACTGGAAGGGCCTAATTCACCCCCAACGAAGACAAGA  BHIS  ATCCCTTOATCTGTGGATCTACCACACACACAAGGCTACTTCCCTGATTAGCAGAACTACACCACGAGGACAAGA  BHIS  AGCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGGTTGAGCCAGAGAAGTTAGAAGAAGCCAACAA  BHIS  AGGAGGAGAACACCAGCTTGTTACACCCCTGTAGACCTGCATGGAATGAACCCGGGAGGAGAAGTTAGAAGAACTGCTGACA  BHIS  BAGAGCTTGACAGCCGCCTAGCATTTCATCACACATGGCCCGAAGAAGTTAGAACAAGAAGTTAGAAGAACTGCTGACA  BHIS  CAGAGTTTGACAGCCGCCTAGCATTTCATCACCATGGCCCGAAGAGCTGCCCGGAGGAGAAGTTACAAGAACTGCTGACA  BHIS  AGCCCTCAGATCCTGCTACAAGGGACTTTCCCCCTGGGGACTTGCCCTGGAGGAGCTGGGGAAGTCGAAGCCT  BAGCCTCAGATCCTGCATATAAAGCAGCTGCTTTTTTGCCTGTACTGGACTCCTCTGTTAGACCAGATCTGAAGCCT  BRIS  BOCAGCTC  BIND  BIND  CCTGGCTAGCTAGCAACCCCACTGCTTAAGCCTCATTAAACCTTGCCTTGAGTTCCTTGAGTACTTCAAGAACTCCTCAGAACCTGAAGCCTCAGAACCTTGAGAACCCTCAGAACCTCCAGAACCTCCAGAACCTCCAGAACCTTGAGAACCCTCAGAACCTTGAGAACCCTCAGAACCTTGAGAACCCTCAGAACCTTGAGAACCCTCAGAACCTTGAGAACCCTCAGAACCTTGATGAAACCTTGCCTTGAGGTCCCTCAGAACCTCCAGAACCTCCAGAACCCTCCAGAACCTCCAGAACACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAACCCTCCAGAAC		***************************************	8471	
AGAAGEACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGC  PULI Bg1 II Aha III		ATCTCGAGACCTAGAAAAACATGGAGCAATCACAAGTAGCAACAGCAGCAGCTAACAATGCTGATTGTGCCTGGCT	8546	
Polypurine Trace 1R  AGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGAACGGGAAGGGCTAATTCACTCCCAACGAAGACAAGA  HIB  TATCCTTGATCTGGATCTACCACACACACACAAGGCTACTTCCCTGATTAGCAGAACTACACCACGGAAGACAAGA  HIB  CAGATATCCACTGACCTTTGGATGGTCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAAGAAGCCAACAA  HIB  AGGAGGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCGGAGAGAAGTTAGAAGAAGCCAACAA  HIB  GAGGTTTGACAGCCGCCTAGCATTTCATCACACTGCAGCTGCATGGAATGGATGACCCGGAGAGAAGTTAGAAGAACTGCTGACA  HIB  TCGAGCTTGACAAGGGACTTTCCGCTGGGGACTTTCCAGGAGAGCGTGCCTGGGCGGGACTGGGAACTGCTGACA  HIB  AGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGAGGCGTGCCTTGAGACCAGATCTGAGCCT  Set I  HIB  HIB  TCTGGCTAGCTAGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTACACCAGATCTGAGCCT  HIB  TCTGGCTAGCTAGCAACCCACTGCTTAAGCCTCCATAAAAGCTTGCCTTGAGTCCTTCA  AGTAGTGTGTGCCCCGTCTGTTGTGTACTCTGGTAACTAGAGATCCCTCAGA  AGTAGTGTGTGCCCCGTCTGTTGTGTGACCTCTGGTAACTAGAGATCCCTCAGA  AGTAGTGTGTGCCCCGTCTGTTGTGTGACCTCTGGTAACTAGAGATCCCTCAGA  AGTAGTGTGTGCCCCGTCTGTTGTGTGACCTCTGGTAACTAGAGATCCCTCAGA	48	AGAAOCACAAGAOQAGGAGGAOGTOGGTTTTCCAGTCACCTCAGGTACCTTTAAGACCAATGACTTACAAGGC	8621	
TATCCTTOATCTOTGGATCTACCACACACACACACGCTACTTCCCTGATTAGCAGAACTACACACAC	110	AGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGA	1676	
HIS AGGAGAACACCACCACCACCACCACCACCACCACCACCAC	110	TATECTTOATCTOTGGATCTACCACACACACAGGCTACTTCCCTGATTAGCAGAACTACACACCAGGGCCAGGGAT	8771.	
HIS OAGGTTTGACAGCCGCCTAGCATTTCATCACATOGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAACTGCTGACA (INS)  HIS TCGAGCTTGCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGCCTGGGCGGGACTGGGGAGTTGCCGAGAACTGCTGACA (INS)  HIS AGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCT (INS)  Set I  HIS GGAGCTC  HING III  POLY(A) SIG.  TCTGGCTAGCTAGGAGACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCA  AGTAGTGTGTGCCCGTCTGTTGTGTGACCTCTGGTAACTAGAGATCCCTCAGA		CAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAAGAAGCCAACAA	8846	
HIS TCGAGCTTOCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTTGCGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGGGACTGGAGCCT    HIS AGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTTGACCCTGTACTGGGTCTCTCTGAGCCTGAACCAGATCTGACCCT   Set I		AOGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCGGAGAGAGA	8921	
MIS AGCCCTCAGATCCTOCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTGCTCTCTGGTAGACCAGATCTGAGCCT  Set I  HIS GGAGCTC  HIS GGAGCTC  TCTGGCTAGCTAGGGAACCCACTGCTTAAGCCTCATAAAGCTTGCCTTGAGTGCTTCA  AGTAGTGTGTGCCCGTTGAGCCTCAGAGATCCTGGAGATCCTCAGAGATCAGAGATCAGAGATCCCTCAGAGATCAGAGATCAGAGATCCCTCAGAGATCAGAGATAGAGATCAGAGATCAGAGATAGAGATCAGAGATAGAGATAGAGATAGAGATAGAGATAGAGATAGAGATAGA	110 18	GAGGTTTGACAGCCGCCTAGCATTTCATCACATGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGACA	2996	•
ACCCCTCAGATCCTOCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTGTGATCAGACCAGATCTGAGCCCTGAGACCTGTACTGGGTCTCTGAGCCTGTAGAGCCTGAGAGCCTGAGAGATCCTGAGAGATCCTGAGAGATCCTGAGAGATCCTGAGAGATCCCTGAGAGAGA		TCGAGCTTGCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCA	9871	
HIR BOGACCTC  HING III  NING III  TOTOOCTAOCTAOCGAACCCACTOCTTAAOCCTCAATAAACCTTOCCTTO		AGCCCTCAGATCCTOCATATAAGCAGCTGCTTTTTTGCCTGTACTGGGTCTCTGTGGTTAGACCAGATCTGAGCCT	9146	
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TETOOCTAGETAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCA  - US  AGTAGTGTGTGCCCGTCTGTTGTGACCTCTGGTAACTAGAGATCCCTCAGA	•	Mind III		
AGTAÖTÖTGTGCCCCTCTGTTGTCTCACTAACTAGAGATCCCTCAGA	<b>B2</b> 1	retogetagetaggaacceactoettaagcetcaataaagcttgeettgagtgettea	9213	
, US	32	AGTAGTOTOTOCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGA		

F1G. 3 (CONT.)



pMR 100 pMR 200

J÷

2+

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**7**+

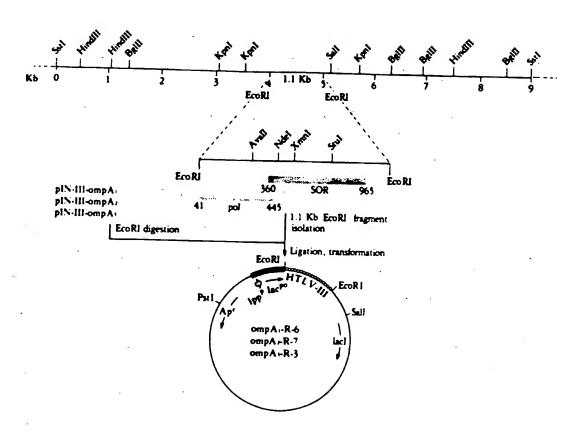
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105+.

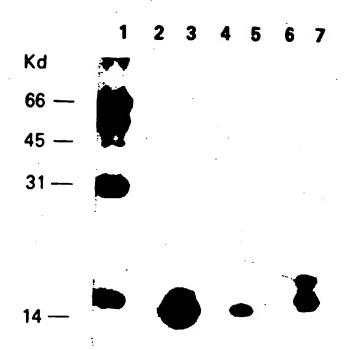
107+

117

F1G.4



F16.5



F/G.6

OMPA SIGNAL PEPTIDE

HTLV - III

EcoRI ▼.

GGAATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTA --ompA3-R-3:----- GCGCAGGCC ----- AlaGinAla

GIyIleProTyrAsnProGInSerGInGIyValValGIuSerMetAsnLysGluLew

EcoRI

GCTGAATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTA ---OmpA2-R-7: ----- GCGCAGGCC

Al aGluPhe Aloginala

EcoRI

OmpAI-R-6:

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AlaAsnSer AloGI nAlo

F1G. 6a

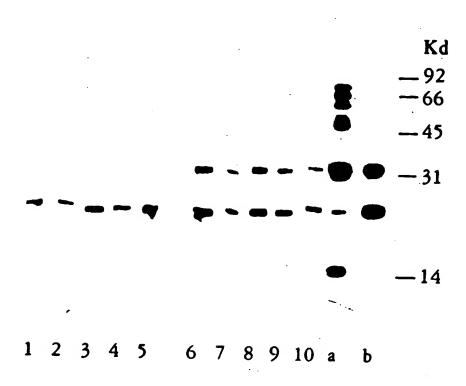


FIG.7

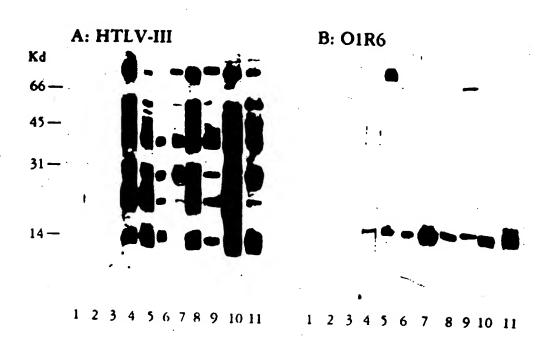
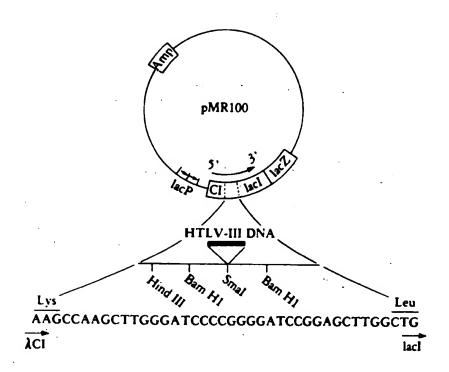
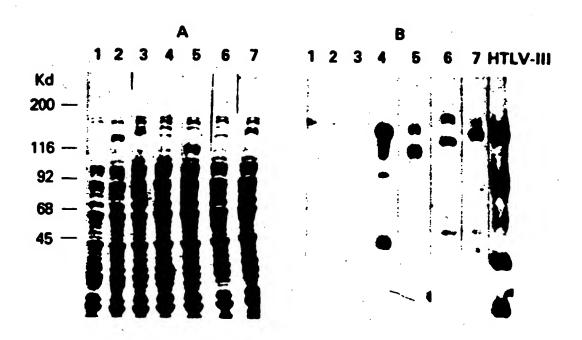


FIG.8



F1G.9



F1G.10



### **EUROPEAN SEARCH REPORT**

Application Number

EP 93 20 0929

Category	Citation of document with i	ndication, where appropriate, ssages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. CL4)
X	SCIENCE. vol. 225, 20 July 1 pages 321 - 323 KALYANARAMAN ET AL. (LAV) in patients w * the whole documen	ith AIDS'	1-4, 10-14, 16-19	C12N15/49 A61K39/21 G01N33/569 C12P21/02 C12N15/62 C12Q1/70
K,P	EP-A-0 138 667 (INS 24 April 1985		1-4, 10-14, 16-19	C12P21/08
1	* the whole documen	t *		
· .	SCIENCE. vol. 225, 31 August pages 927 - 929 ARYA ET AL. 'Homolo AIDS-associated vir human T-cell leukem * the whole documen	us with genomes of ia viruses!	5	
*	EP-A-0 178 978 (INS 23 April 1986 * page 17 *	TITUT PASTEUR/CNRS)	5-6,9,15	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
E .	EP-A-0 173 529 (THE AMERICA) 5 March 1986 * the whole documen		5,9,15	C12N C12P A61K G01N C12Q
		·		
	The present search report has b	·		
٦	Place of search THE HAGUE	Data of completion of the search 21 MAY 1993		CUPIDO M.
X : par Y : par doc	CATEGORY OF CITED DOCUME ticularly relevant if taken alone ticularly relevant if combined with an unsent of the same category innlogical background	E : earlier patent after the filir other D : document cit	ciple underlying the document, but public date and in the application of for other reasons	lished on, or

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